

3. Plant-sugar feeding and vectorial capacity

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Abstract

Sugar feeding is a common behaviour of male and female mosquitoes, sand flies, and other Dipteran vectors. In some species it is essential to one or both sexes; in others it is facultative. Even among females of anthropophilic species that are predisposed to a diet of frequent blood meals sugar is often taken, depending on internal state and opportunity. This opportunism is expressed as an increased likelihood of feeding on nectar when access to blood and oviposition sites is limited. Newly emerged *Anopheles gambiae* females sometimes show a preference for sugar before mating even when blood hosts are available, likely depending both on the strength of plant and animal kairomones and on the attractive qualities of each. Incorporation of sugar in the diet by mosquitoes affects certain components of their vectorial capacity. Environmental conditions, such as bed net coverage and abundance of nectar sources, will affect the extent to which mosquitoes feed on sugar. If the effect of sugar on vectorial capacity is significant, these conditions will impact transmission rates of vector-borne diseases and should be included in epidemiological models. Vectorial capacity is pulled in opposing directions by sugar feeding, through its effect on the two most important components, survival and biting rate. Survival of females feeding on sugar and blood is greater than that of females restricted to a blood-only diet, according to the vast majority of studies, whereas biting rates usually are depressed when sugar is available, but field evidence is scarce. Vector density results from survival and fecundity. Most studies on vectors suggest that although fecundity per gonotrophic cycle is enhanced by sugar feeding, long-term reproductive fitness in anthropophilic species is slightly depressed. Vector competence appears to be negatively affected by sugar feeding. In certain cases plant nectar contains factors that inhibit development of the parasite in the vector. More common may be positive effects on the vector's immune response, but this appears to depend heavily on the host-parasite system, condition of the vector, and possibly genotype-by-environment interactions. Estimating the combined effect of these factors at different levels of sugar intake remains difficult at this point, but an overall impression is that vectorial capacity is somewhat decreased in environments where sugar is readily accessed. Sugar feeding behaviour can be exploited for control, the most promising methods employing sugar solutions combined with attractants and oral insecticides for direct control and attractive phytochemicals for surveillance. Main questions facing both approaches are their suitability in verdant areas where attractants will compete with a diverse flora. For females of anthropophilic species in settings with abundant blood hosts, the question may be whether populations can be effectively suppressed by targeting male mosquitoes.

Keywords: nectar feeding, vectorial capacity, fitness, novel control methods

Introduction

Overviews of insect-vector sugar feeding (Downes 1958, Foster 1995, Yuval 1992) identified important gaps in our understanding of the process and its implications. One particularly important gap was the influence of available plant sugar on vector populations: whether it is a limiting resource, so that its restriction can affect reproductive success and survival, and therefore the sustained density of adults, i.e. the carrying capacity of the environment. Another gap, equally important, was the effect of plant sugar on pathogen transmission, including how the availability of sugar can affect vectorial capacity by altering vector competence or by changing biting

frequency and survival. Because density of adults also contributes to vectorial capacity, these two gaps in our knowledge are part of the same question for disease ecologists: is plant-sugar feeding by vectors a critical component of pathogen transmission?

Investigations of sugar feeding vector biology, both in the laboratory and in the field, made since those comprehensive reviews, are beginning to provide details that can fill these gaps. This review will focus mostly on newly published work and on aspects of the plant-vector topic not previously discussed. For the earlier literature supporting conclusions and generalizations about mosquito sugar feeding, summarized in the present review but not fully referenced, the reader is referred to Foster (1995). For malaria transmission in particular, the possibility that sugar feeding by *Anopheles* mosquitoes may be an essential component of the epidemiological process is gaining wide recognition (Ferguson *et al.* 2010). Its importance for leishmaniasis transmission by phlebotomine sand flies in desert regions also is strongly supported. Experiments in both disease systems are providing direct evidence for plant-sugar's pivotal role in vector biology and offering ways to manipulate the connection between plant and vector to weaken or eliminate pathogen transmission, either by itself or as a valuable component of integrated control (Beier *et al.* 2008; Shaikat *et al.* 2010).

Taxa involved and evidence

Taxa covered

Most blood-feeding Diptera also ingest plant sugar. For this reason, it is accurate to say that they have two types of hosts: vertebrate animals and vascular plants. These dipterans include the blood-feeding species of the families Culicidae, Ceratopogonidae, Simuliidae, Psychodidae, Tabanidae, Rhagionidae, and some blood-feeding Muscidae. Notable exceptions are the tsetse flies (Glossinidae) and the ectoparasitic pupiparous dipterans (Hippoboscidae, Streblidae, and Nycteribiidae). For a few poorly known haematophagous flies, e.g. Corethrellidae and Carnidae, the role of sugar feeding has not yet been established. No other haematophagous insects are known to take plant sugar. These blood feeders include the Siphonaptera, among Hemiptera the triatomine Reduviidae, the Cimicidae, and the Polyctenidae, and among Phthiraptera the anoplurans, amblycerans, and rhynchophthirines. This also appears to be true of all haematophagous Acari. So far, the great majority of sugar feeding investigations have targeted mosquitoes (Culicidae) and the phlebotomine sand flies (Psychodidae). However, recent important plant-related studies include horse flies and deer flies (Tabanidae), black flies (Simuliidae), biting midges (Ceratopogonidae), and stable flies (Muscidae).

Plant food sources and composition

The sugar feeding haematophagous Diptera obtain their sugar from a variety of plant sources, most commonly floral and extrafloral nectar and honeydew; the latter is plant-derived but homopteran-produced. Other sources are damaged or decaying fruit and seeping sap from plant wounds. Typically, these flies direct the ingested vertebrate blood straight to the midgut, where most digestion and all absorption occurs. They shunt all but the smallest sugar meals to the foregut diverticula, blind sacs where sugar is stored prior to being doled, a little at a time, into the midgut for digestion and absorption. Most of a sugar meal is stored in the large ventral diverticulum, the crop. Although the sugars sucrose, fructose, and glucose are the primary constituents of nectar, minor sugars also occur, and various oligosaccharides are common in honeydews. Glycosidases in mosquito and sand fly saliva (Jacobson and Schlein 2001, James and Rossignol 1991, Marinotti

and James 1990) and midgut (Billingsley and Hecker 1991, Jacobson and Schlein 2001, Souza-Neto *et al.* 2007) cleave sucrose into its constituent hexoses: fructose and glucose. In addition, plant-sugar meals usually contain amino acids and are considered to be part of the flower-pollinator and extrafloral gland-mutualist syndromes (Shuel 1992). The amino acids by themselves are insufficient to stimulate or support mosquito egg development, but they do appear to promote survival (Eischen and Foster 1983, Jones *et al.* 1985, Vrzal *et al.* 2010) and also may serve as a flight substrate (Scaraffia and Wells 2003). Many other nectar constituents have been found, some presumably nutritional, others distasteful or toxic.

Tissue piercing

Healthy plant tissue is sometimes pierced, and sugars and other nutrients are then extracted from phloem sap or tissue fluids. Tissue feeding has been examined most extensively and intensively in desert sand flies, where it appears to be essential to their survival. In mosquitoes, this phenomenon has been reported many times in the literature, yet it has not been explored intensively, and its significance for them globally remains unclear. The most recent evidence for mosquito and sand fly tissue feeding comes from studies in Israel, where calcofluor-stained cellulose particles have been detected in the midgut (Junnilla *et al.* 2010, Müller and Schlein 2005, Müller *et al.* 2010b, Schlein and Jacobson 1999, Schlein and Müller 1995), and chloroplast DNA has been identified (Junnilla *et al.* 2010). Amylase activity has been detected in many haematophagous flies (reviewed by Gooding 1975), including sand flies and mosquitoes, and amylase gene expression has been explored in mosquitoes (Grossman *et al.* 1997). In phlebotomine sand flies it is used to digest starch granules obtained during plant tissue feeding (Jacobson and Schlein 2001, Jacobson *et al.* 2001, Ribeiro *et al.* 2000). The fluid ingested by sand flies during plant piercing is transferred directly to the midgut (i.e. in the 'blood-feeding mode'), rather than being shunted into the crop (Schlein and Warburg 1986). The presence of amylase in mosquitoes and other haematophagous Diptera suggests that starch, derived from tissue feeding, is part of the diet. In both sand flies and mosquitoes it is reported to be particularly common during seasons and localities when and where sugar sources are rare and plants are under heat and water stress (e.g. Müller *et al.* 2010c, Schlein and Jacobson 1999, Schlein and Müller 1995). Tissue piercing may provide water as well as sugar, but it also affects *Leishmania* infections in sand flies (Schlein and Jacobson 2001) (see below).

Methods for evaluating plant feeding

The evidence for sugar feeding in the field, and much of what we know about it, comes either from direct observations of insects on plants or from chemical tests of gut contents. Observations of vector behaviour on plants are sometimes difficult to interpret, because landing, aggregating, and even probing do not necessarily result in ingestion of sugars. Furthermore, the failure to observe plant-feeding behaviour, and therefore deduce its absence, is notoriously misleading. Sugar feeding often occurs rapidly and over broad periods of the insect's activity period, spread over broad sweeps of a landscape that supports a vector's host plants. This is unlike blood-feeding behaviour, which tends to be concentrated on relatively scarce hosts and consequently is more obvious to the human observer.

Chemical tests are less susceptible to sampling biases than direct observations. The one that revolutionized sugar feeding studies of vectors is Van Handel's cold-anthrone test (Van Handel 1967, 1972) for fructose, a plant sugar not synthesized *de novo* within the insect. Other methods for detecting fructose have been developed that are reported to give greatly increased sensitivity (Nunes *et al.* 2008, Somani *et al.* 1987). Simple chromatographic methods also have proved

satisfactory for detecting undigested sugar meals, provided that they distinguish between plant-derived and metabolically generated sugars (Laarman 1968, Magnarelli and Anderson 1977, Magnarelli 1978, 1979, 1980, Nayar 1978, Watanabe *et al.* 1973). Particularly useful have been thin-layer and gas chromatography that identify sugars distinctive to honeydew, as opposed to floral and extrafloral nectars (Burgin and Hunter 1997a,b,c, Burkett *et al.* 1998, 1999, Hunter and Ossowski 1999, Janzen and Hunter 1998), or that can help determine the likely plant-host species by their sugar ratios (Hamilton and El Naiem 2000, Manda 2007a). Finally, evidence for the penetration of undamaged plant tissue, such as leaves and stems, can be deduced from the presence of dyes and cellulose (e.g. Müller *et al.* 2010b, Schlein and Jacobson 1999, Schlein and Müller 1995), and the plant-host species can be identified from chloroplast DNA (Junnala *et al.* 2010). The principal problem we confront with chemical tests of gut contents is that sugars and other materials disappear either gradually or rapidly, depending on the amount consumed and rates of digestion and egestion in each species according to temperature and the individual's physiological status. Many negatives will be recorded, even among species that plant-feed at frequent intervals. So the tests provide only an approximation of the proportion of vectors that have fed on plants during a particular period of time.

An underutilized low-bias approach to determine the identity of plant hosts is the identification of pollen grains on the body or mouthparts, or in the gut. Pollen has been used effectively as an indicator of specific plant visits by the tabanids *Tabanus* and *Chrysops* (Magnarelli 1979) and the stable fly *Stomoxys* (Jarzen and Hogsette 2008, Tseng *et al.* 1983). Pollen also has been found on a wide variety of mosquito species, some of which are implicated as pollinators. Pollen information is limited by the uncertainty of the time that the pollen was acquired. In addition, some pollen grains are too large to be incorporated in pollen-contaminated nectar meals of insects with narrow food tubes or are held in pollen-transfer devices that do not adhere to the bodies of small nectar thieves. Also, pollen will not account for a vector's visits to non-floral sugar sources, and cross-contamination of pollen between plant species conceivably may lead to false conclusions.

General features of plant feeding behaviour

Autogeny and diapause

A good baseline of knowledge about sugar feeding behaviour in mosquitoes exists, derived from a variety of lab and field studies. In the case of females of autogenous species, a sugar meal often is necessary for the development of the first batch of eggs (O'Meara 1985, 1987). Even among anautogenous mosquitoes there are species that rarely or never seek blood until they take at least one sugar meal (Briegel *et al.* 2001, Hancock and Foster 1997, 2000, Renshaw *et al.* 1994, 1995). Sugar feeding frequency may diminish in females of some anautogenous species, once insemination is achieved and blood feeding commences, whereas in males it remains constant throughout life. On the other hand, where winters are severe, females that have entered a state of adult diapause prior to overwintering either do not take blood at all (*Culex*) or take non-ovigenic blood meals close to their hibernacula (

Anopheles). However, *Culex* females entering diapause up-regulate genes for fatty acid synthase (Robich and Denlinger 2005, Sim and Denlinger 2009), and indirect evidence indicates that they feed on sugar frequently prior to entering hibernacula (Bowen 1992a, Jaenson and Ameneshewa 1991). During this period they accumulate large reserves of fat before foraging becomes impossible. Where winters are milder, some sugar feeding may occur among diapausing *Culex* populations

throughout the winter (e.g. Reisen *et al.* 1986), perhaps explaining the sporadic expression of the fatty acid synthase gene throughout simulated hibernation.

Food utilization

Ingestion of sugar is directly correlated with flight range, and sugar can be consumed directly as a flight-energy substrate. Alternatively, it may be converted to glycogen for storage in the insect's fat body and flight muscles. Although previously thought to be used primarily for survival, stored lipid derived from either blood or sugar also can serve as a flight substrate in *Anopheles gambiae* Giles, which has an exceptional ability to mobilize lipid during long flights (Kaufmann and Briegel 2004), likely involving adipokinetic hormone. There is evidence that even amino acids derived directly from the blood meal may be used in flight in *Aedes aegypti* Linnaeus (Scaraffia and Wells 2003).

Timing and frequency

The first adult food of both sexes of anautogenous species is likely to be plant sugar, and both sexes continue to take sugar throughout their reproductive lives. Sugar feeding by females appears to be least likely to occur when they are digesting a blood meal and most often when gravid or prior to the next blood feeding. But there are many exceptions to this, depending both on species and circumstances. Sugar feeding has a characteristic time, or times, in the diel activity cycle. Recent studies have started to elucidate the molecular basis of this rhythmic behaviour (e.g. Rund *et al.* 2011). Cycles of sugar feeding often share a general activity rhythm with other behaviours, so that the phases of different behaviours are the same or nearly so. A field study of *An. gambiae* by Müller *et al.* (2010b) demonstrated that the times of sugar seeking and blood seeking, though to some extent overlapping, occurred in distinctly different parts of the diel cycle: attraction to sugar baits occurred mainly early in the night, with a second peak shortly before dawn, whereas attraction to blood-host odour occurred mainly in the second half of the night, in accord with landing or biting rates of other studies of this species.

Average sugar feeding frequency is difficult to infer from field data, because of strong temperature fluctuations and narrow periods when feeding occurs. Rough estimates are based on the time for all individuals to digest naturally acquired nectar meals completely and on the fraction of resting individuals that contain a meal still in some stage of digestion. By extrapolation, the time spent without sugar is calculated, and the total time with and without sugar provides the sugar feeding interval. Typical values indicate that males in the field may take sugar every 1-2 days, whereas mature females of anthropophilic species may take it as infrequently as every 6-9 days. These species are less dependent on sugar for successful reproduction, flight, and extended life in the laboratory (Fernandes and Briegel 2005, Harrington *et al.* 2001, Kaufmann and Briegel 2004), and they less often contain undigested sugar meals in the field (as explained below). Females of typical zoophilic species, by contrast, often contain undigested sugar and probably feed on sugar at least as frequently as on blood, i.e. every 3-4 days. Without sugar, they can die rapidly, despite frequent access to animal or even human blood (Fernandes and Briegel 2005, Nayar and Sauerman 1975, Wittie 2003).

Limited and limiting availability in the field

In laboratory cages, sugar availability clearly alters survival and reproduction. Evidence from the field is much harder to come by, primarily because of difficulties in measuring the accessibility of sugar in nature. Field samples support the general notion that more sugar feeding occurs

when more sugar sources, or just more plants, are available (Martinez-Ibarra *et al.* 1997, Müller *et al.* 2010d). These studies suggest the hypothesis that plant sugar is a limited, and potentially fitness-limiting, resource. The underlying assumption is that up to some unknown point, it is advantageous for vectors to take more sugar if they can find it. In several studies (e.g. Gadawski and Smith 1992, Hocking 1953, 1968), vector population density was low if fewer preferred nectar sources were available, or declined seasonally when the sugar sources declined. Some of the best evidence for this effect comes from the few studies of sand flies and mosquitoes in isolated areas in which a suspected plant host either was marked with a sugar-baited dye or was sprayed with insecticidal bait (Müller and Schlein 2006, Schlein and Müller 2008). Another approach was to compare the reproductive performance of cohorts in mesocosms with and without sugar sources (Stone *et al.* 2009), or to measure the density and survival of populations of vectors in isolated areas that appear to differ only in the availability of certain preferred plant hosts (Gu *et al.* 2011). In the last case, a drastic difference in survivorship and biting frequency has been attributed to the presence or absence of a single species of host plant (See more in the Section 'Vectorial capacity').

Plant-host preference

Mosquitoes and other vectors collected while standing, crawling, or probing on a variety of plants in bloom appear to show preferential attraction to certain plant species, because they occur disproportionately on those plants. This apparent degree of host specificity is reinforced by the many anecdotal observations that link an insect to only one or a few plant species. Another source of evidence for plant-host selectivity is the ability of vectors to obtain fluids, secretions, or nutrients from only a small subgroup of the species in a plant community. For example, Abdel-Malek and Baldwin (1961) were the first to suggest selective removal of sugar-bearing plants as a means of control (see Section 'Selective removal of plants'). They found that *Ae. aegypti* and three indigenous Canadian mosquitoes fed on only three of 24 native plant species offered to them. A study in a natural setting in Egypt likewise revealed that *Anopheles sergentii* Evans males fed on a very select number of plants, and the presence of those plants predicted the presence of larvae in nearby pools. Furthermore, field collections of males were successful on these plants, whereas very few *An. sergentii* were collected from other plants (Abdel-Malek 1964). Similar laboratory experiments demonstrating differences in mosquito and sand fly ingestion and survival on various plant species have been conducted by Patterson *et al.* (1969), Schlein and Warburg (1986), Alexander and Usma (1994), Gary and Foster (2004), Impoinvil *et al.* (2004) and Manda *et al.* (2007b).

Even investigators who take into account the relative availability of all possible host plants can misinterpret insect aggregation – possibly the result of behavioural arrest – as attraction. Recent sand fly and mosquito experiments have eliminated some of this bias and confirm that vectors have plant preferences. These experiments have used plant-baited or plant-associated traps and resting sites (Schlein and Yuval 1987, Müller and Schlein 2004, Gouagna *et al.* 2010), selective dye-marking or insecticide treatment of plants in the field (Schlein and Jacobson 1994, Schlein and Müller 1995, 2008, Müller *et al.* 2010b, Müller *et al.* 2011), radioactive tagging of plants (Abdel-Malek and Baldwin 1961, Abdel-Malek 1964, Patterson *et al.* 1969), and wind-tunnel olfactometers (Gouagna *et al.* 2010). The existence of strong differences in attraction of *An. sergentii* to specific plants was convincingly demonstrated by miniature CDC-light traps baited with branches of potential plant hosts (Müller and Schlein 2006). Müller *et al.* (2010a) also found evidence for differences in attraction of *An. gambiae* to fruits and flowering plants in Mali using wire-mesh glue traps surrounding 26 plant species and 26 kinds of fruits and seedpods. Flowering plants

were considerably more attractive than fruit, and over all, nine out of the 26 plants tested were considered attractive, with only minor differences between males and females.

A completely unbiased form of evidence for selective plant-feeding (i.e. the host-utilization rate) in nature must come from the guts of random samples of vectors collected in the field. These kinds of data, when combined with knowledge of the proportions of different plant species available to the vector, provide a measure of plant preference (i.e. the forage ratio or feeding index). Rigorous studies of this kind have yet to be conducted. However, chromatographic profiles of sugars in *Phlebotomus orientalis* Parrot sand flies in the Sudan demonstrated the relative importance of fruit and honeydew (Hamilton and El Naiem 2000) and in several species of mosquitoes (Burkett *et al.* 1999), black flies (Burgin and Hunter 1997a,b,c), deer flies and horse flies (Hunter and Ossowski 1999, Janzen and Hunter 1998). Chloroplast nucleotide sequences showed that during the dry season *An. sergentii* mosquitoes mainly tissue-fed on three succulent species that formed less than 1% of the vegetation (Junnala *et al.* 2010).

Obligatory vs. facultative nature of sugar feeding

Anthrophilic and generalist species

Among mosquito species there appears to be a continuum of female reliance upon sugar feeding. At one extreme, *Toxorhynchites* females feed exclusively on plant sugars, relying for egg production on the protein reserves from the larval stage. At the other extreme, where human blood is readily available, certain species have been reported to contain plant sugar infrequently or rarely (Beier 1996, Costero *et al.* 1998, Edman *et al.* 1992, Gillies 1968, Spencer *et al.* 2005). Females of these species may rely on blood entirely, often feeding multiple times per gonotrophic cycle (e.g. Beier 1996, Braks *et al.* 2006, Edman *et al.* 1992, Foster and Eischen 1987) or having overlapping gonotrophic cycles (Briegel and Hörler 1993). Nonetheless, such females readily feed on sugar in the laboratory, and a modest proportion can be found to contain sugar in the field.

Species for which facultative sugar feeding has been proposed are *An. gambiae* and *Ae. aegypti*. They occupy a specialized niche and share a number of characteristics, in particular anthropophily and endophily, that predispose them to a diet limited to blood. Due to their tendency to rest indoors after feeding on blood, a female's energy requirement for flight is limited to that needed for seeking hosts, mates and oviposition sites; the energy required to locate any of these, near domiciles, probably is small or negligible. The mating behaviour of *Ae. aegypti* is notable in that swarming and mating occur around the blood host, instead of above inanimate swarm markers. Yuval (2006) proposed that this behaviour may be an adaptation to dispersed and non-synchronous emergence of adults. Alternatively, it may be simply a result of intense intrasexual selection. *An. gambiae* do mate in conventional swarms, but the flight energy a female might expend to locate the swarm, mate, and return to a resting site, has not been examined. The discovery that mating in this species sometimes occurs indoors in West Africa (Dao *et al.* 2008) implies that in some areas the energetic costs of mating and host seeking likewise may be small and overlapping, as in *Ae. aegypti*.

The lower incidence of sugar feeding, and higher rate of multiple blood feeding per gonotrophic cycle is likely one of the reasons both *Ae. aegypti* and *An. gambiae* are such efficient vectors of human disease. These characteristics may have evolved in response to an oddity of human blood composition. One of the amino acids essential for vitellogenesis, isoleucine, is notably limited in human blood compared to that of other vertebrates (Briegel 1985, Dimond *et al.* 1956, Lea *et al.* 1958). Consequently, a smaller proportion (up to 30% less) of each blood meal can be used for

gametic functions, allowing for a greater investment of blood-meal carbon to somatic functions. Kaufmann and Briegel (2004) provided an elegant example of this differing physiological reliance on sugar by comparing the flight distances of *An. gambiae* and *Anopheles atroparvus* Van Thiel when fed on sugar or blood. *An. gambiae* females mobilized their lipid reserves for flight and were capable of flying similar distances after being fed on sugar or on two blood meals. The more generalist blood feeder *An. atroparvus*, in contrast, did not use lipids to fuel its flight and achieved greater flight distances when fed sugar.

Mosquitoes feeding on blood hosts with higher isoleucine contents may thus be expected to have a higher reliance on sugar for the maintenance of energetic reserves. Whether prior sugar feeding by generalist mosquitoes, in turn, affects blood-host choice is not known. Even among anthropophilic species, under certain circumstances non-human animals may form larger proportions of their blood meals. Unclear at this point is whether feeding on non-human animals elevates their tendency to take sugar meals. This notion is supported by a comparison among experiments that have given markedly different survival and fecundity results, depending on whether the host was human, bird, or rodent. One example from a single study demonstrated that *Ae. aegypti* females, fed on human blood, had superior lifetime fecundity when sugar was absent; but when fed on mouse blood, fecundity was higher when sugar was present. Individual mouse blood meals, supplemented by sugar, generated by far the largest egg output (data courtesy of L. Harrington to W.A.F.). Yet, per mg, mouse blood alone was significantly less productive than human blood with sugar (Harrington *et al.* 2001). Lifetime survival showed a similar relationship except that there was no difference in survival on human blood, with or without sugar access. Studies on *Aedes albopictus* Skuse, which more often takes animal blood meals in nature, demonstrated that on human blood, females without sugar had moderately reduced survivorship (Braks *et al.* 2006). But on bird blood, females without sugar had drastically shortened lives (Xue *et al.* 2010).

Field evidence

The field evidence for a low incidence of plant-sugar feeding by anthropophilic species is not unequivocal. Of particular concern for the interpretation of fructose-positivity rates of field-collected females, in addition to those previously described, is the likelihood of collecting host-seeking or blood-fed females in indoor resting catches, potentially under-representing females in states that may be more inclined to sugar feed. Compounding these issues is the high variation reported in fructose rates between geographical areas and seasons, the main question being whether this variation is best ascribed to differences in plant community composition and abundance, or to the presence and availability of preferred blood-hosts.

Several field and laboratory studies provide insight into the facultative nature of sugar feeding of *Ae. aegypti* and *An. gambiae* and its variation across different habitats. For instance, wild *Ae. aegypti* were collected in a rural village in Thailand and tested for the presence of sugar during both the wet and the dry season (Edman *et al.* 1992). Seasonality did not affect sugar positivity, and only 3% of females were sugar positive, vs. 35% of males, demonstrating that sugar sources were at least available. In another study in Thailand seasonality did affect sugar intake of females (Spencer *et al.* 2005). Further evidence of limited sugar intake was obtained by collecting males and females inside houses in San Juan, Puerto Rico (Van Handel *et al.* 1994). Only 2% of the collected females contained fructose, and all females that were blood-fed or gravid were fructose negative. This could not be explained by the absence of sugar sources, because houses, patios and backyards typically contained a large number and variety of plants that might serve as nectar sources and 51% of males were fructose positive. The results from these field collections are in

stark contrast with sugar-positivity rates of females collected at a rural site (a tire dump) near Vero Beach, Florida. There, collected females contained no eggs or blood, and 74% contained fructose, while 63% of males did so (Van Handel *et al.* 1994). The authors attribute this striking difference to the difference in blood-host abundance between an urban centre with a dense human population and the rural site where humans are rare. A different field study did attribute the extent to which *Ae. aegypti* feed on plant nectar on the abundance of plants (Martinez-Ibarra *et al.* 1997). More females in the outskirts of Huixtla, Chiapas, Mexico, were sugar-fed (21%) than in the midtown area (8%). There was no difference in number of inhabitants per house between these areas, but there was a significant difference in the number of flowering plants between the areas. Besides the difference in abundance of plants, sugar feeding may have been affected by the availability of specific plants, because bougainvillea and hibiscus occurred in 71% and 53% of the houses with sugar-positive mosquitoes, though it is not clear whether these plants were also more common in houses with sugar-positive mosquitoes than in houses without (sugar-positive) mosquitoes. Thus, there are conflicting reports in the literature about whether sugar feeding by this species is driven by absence of blood-hosts or presence of adequate sugar sources.

Field evidence for the use of sugar by *An. gambiae* s.s., and the effects of environmental conditions on this behaviour, are scarcer than they are for *Ae. aegypti*. Most field evidence suggests that sugar feeding is rare, although a study by Laarman (1968) suggests that feeding on sugar is a normal component of *An. gambiae* behaviour. Muirhead-Thompson (1951) considered sugar to be an unnatural food source for this species, and Gillies (1968) found little evidence of sugar feeding in females collected indoors, based on the absence of fluid in their crops. McCrae (1989) suggested the habitats of *An. gambiae* were characterized by a paucity of sugar sources, but did observe *An. gambiae* s.l. feeding on the extra-floral nectaries of *Avena macrostachya* (personal communication to W.A.F.). Indoor-resting catches and indoor-biting catches in Kisian and Saradidi, Kenya, revealed 'surprisingly low' proportions of female *An. gambiae* s.l. and *An. funestus* Giles with detectable fructose (Beier 1996).

Laboratory studies on the blood/sugar choice

Field observations are not easy to interpret in terms of how likely a female is to feed on sugar at particular times in her life. A number of laboratory studies provide insight on this subject and suggest that while *An. gambiae* may indeed not be an obligate sugar feeder, sugar is a viable option for their first meal (Foster and Takken 2004), and is increasingly used later in life where blood hosts or oviposition sites are less accessible or more distant from one another (Gary and Foster 2006). Based on the finding that *An. gambiae* would feed on a human 24 hr after emergence, and that non-oogenic females were able to convert as much protein and lipid into maternal reserves as oogenic females transferred to egg yolk, Fernandes and Briegel (2005) suggested that this species may be opportunistic in terms of its feeding behaviour, i.e. both sugar and blood meals allow for rapid increases in reserve levels, and whichever is encountered first may be taken. This idea was supported by studies in mesocosms that showed that the pre-mating meal taken by this species favours sugar, but a proportion does feed on blood instead, and a greater proportion does so in the absence of sugar sources (Stone *et al.* 2011). Sugar-related and human-related volatiles thus clearly compete, and the determination of the initial meal choice may depend on both the strengths of the competing stimuli and their qualities.

In *Culex nigripalpus* Theobald, a mosquito that strongly prefers to feed on sugar before feeding on blood, this situational decision-making was demonstrated by Hancock and Foster (1997). In a wind tunnel choice test between sugar sources (honey) and blood (small birds), the response

to either food increased with increasing number of dishes of honey or the number of birds. A comparable study on *An. gambiae* in a mesocosm showed that blood-host presence (a human sleeping in the mesocosm throughout the night, or being available only for one hr per night) and female size, but not abundance of *Senna didymobotrya* (Fresen) Irwin and Barneby, affected the sugar/blood choice of 1-day old females, which strongly favoured blood in this case (Stone et

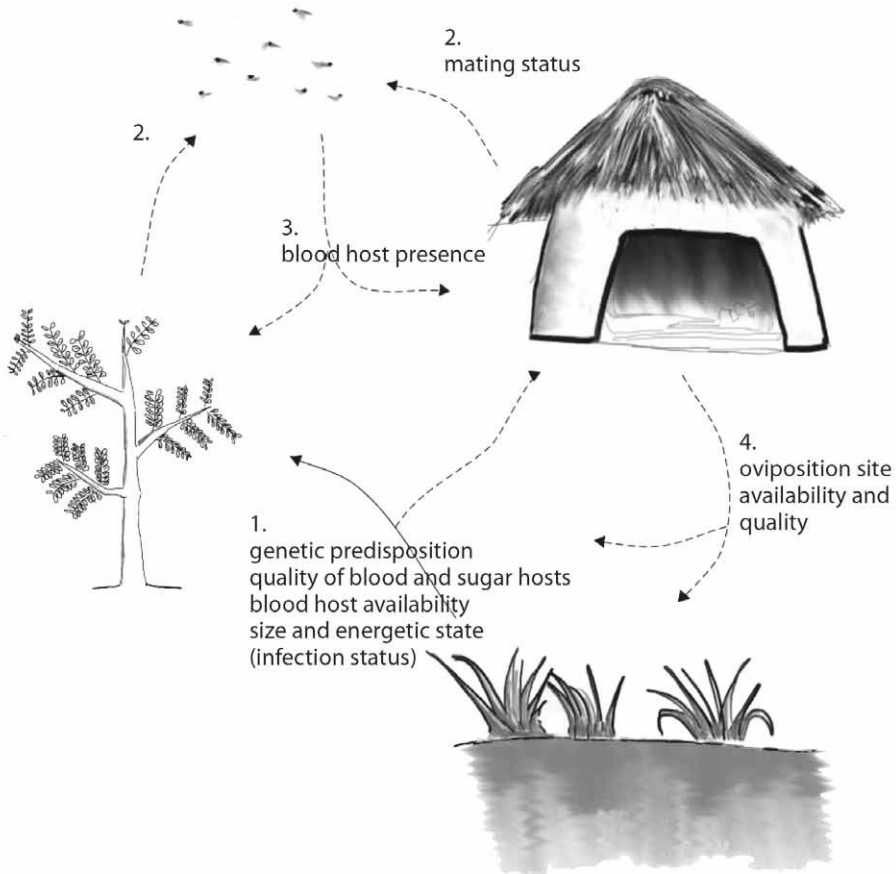


Figure 1. Life choice diagram for *Anopheles gambiae* females, highlighting the behavioural components, the likely sequence in which they move through reproductive cycles, and factors informing the decisions between behaviours. (1) After emergence, or oviposition, females face a choice between seeking blood or nectar. (2) Depending on their mating status, females may seek out a male swarm. (3) Females will then seek a gonoinactive (= 'pre-gravid') or gonoactive blood meal, unless blood hosts are limited, in which case the likelihood of sugar feeding increases. (4) After a gonoactive blood meal, gravid females will oviposit, unless suitable sites are unavailable, in which case the likelihood of sugar feeding increases.

al. 2012). This promotes the notion that females of this species are, rather than obligatory sugar

feeders, opportunistically inclined to use this resource at various points in their lives, depending on environmental conditions and resource accessibility and quality (Figure 1).

Sugar feeding by mosquitoes, according to optimal-foraging theory

Whether or not an animal should include in its diet an item with a particular energetic gain, probability of encounter, and handling time cost, is a question of classic foraging theory (Stephens and Krebs 1986). An assumption inherent to this theory is that animals will maximize their rate of energy input, which is then considered a proxy for fitness. As most animals have limited time-budgets and operate under constraints, and thus face trade-offs, using energy intake as a proxy for fitness is not always informative or free from error. An extension of classical foraging theory, dynamic state variable modelling (Clark and Mangel 2000, Mangel and Clark 1988), employs a fitness function that allows the maximization of a more relevant parameter. Typically this will be lifetime reproductive output. Theories based on these ideas could provide relevant insight into how the tendency to sugar-feed in certain mosquito species depends on environmental factors (e.g. resource availability). Additionally, mosquitoes in different behavioural states (age, mating status, body size, energetic reserve level, diapause status, etc.) differ in their tendencies toward taking sugar. To date, general theories applied to this have been developed (Roitberg and Friend 1992, Roitberg *et al.* 1994), and one study has investigated when to sugar-feed for the specific case of *An. gambiae* (Ma and Roitberg 2008). The main predictions from their model are that after emergence, a female is likely to take a sugar meal to increase her reserves before moving indoors to seek a blood host. Around houses, she seeks sugar only when energy levels become very low, and frequency of sugar feeding becomes negligible with increasing blood host availability. Following oviposition, she again commonly takes sugar – unless her energy reserves are high, in which case she returns indoors immediately. Longevity and fecundity both increase with increasing availability of sugar hosts outdoors and blood hosts indoors, whereas presence of peridomestic sugar hosts has negligible effects on these parameters. Several aspects that may be relevant to these predicted feeding choices have not yet been theoretically explored, such as the size or mating status of females. Another realm of questions that would benefit from model-driven hypotheses relate to how female mosquitoes infected with parasites may be expected to modify their feeding decisions (see Section ‘Vector competence’ below).

Vectorial capacity

Components of vectorial capacity

Vectorial capacity (C) is a simplified measure of a vector’s power for pathogen propagation. It is expressed as the total number of new vertebrate cases of an infection that can arise directly from one original infection in a particular environmental setting, due to the insect or other carrier in question. It is a subset of the equations originally developed by Ross (1910) and MacDonald (1957) to provide a quantitative epidemiological description of pathogen transmission and spread among humans by anopheline mosquitoes, in terms of the reproduction rate of cases of malaria. Vectorial capacity was introduced by Garrett-Jones (1964) and Garrett-Jones and Shidrawi (1969) as a means of singling out the vector components useful to entomologists in evaluating the potential ability of a particular insect population to spread a disease. In its simplest form, vectorial capacity is a function of female density relative to humans (m), biting frequency (a), survival rate (p), and duration of the extrinsic cycle (n), according to this simple expression:

$$C = \frac{ma^2p^n}{-\ln(p)}$$

Vectorial capacity is most sensitive to changes in survival rate (p) and biting frequency (a) of females. Because of their magnification by powers of n and 2, respectively, even small changes result in large effects.

Vectorial capacity is oversimplified (Dye 1992), because in its original form it assumes perfect vector competence (see below). Yet in practice, even the simplest version of vectorial capacity is difficult to employ, because its parameters are not easy to measure. Still, this formula has tremendous heuristic value. It allows an entomologist to focus on control efforts that are likely to achieve maximum effect. For example, it becomes clear that the density of vectors, which can be manipulated by larval suppression, whether by insecticides or source reduction, is not nearly as important as reduced adult survival, whether by residual insecticide applied to resting sites, insecticide-treated bed nets, or reduced availability of sugar sources. It also shows, in a quantitative way, which coefficients are most important to measure and worth the effort to investigate by detailed study.

Thus, to increase its heuristic utility, researchers have suggested including an approximation of vector competence (b) as a factor in the numerator of vectorial capacity. In addition, to provide a more realistic value for the probability of survival, researchers have introduced survival rates as various sorts of non-linear functions (e.g. Bellan 2010, Dawes *et al.* 2009, Styer *et al.* 2007b), so that the probability of death may be high or low early in adult life, decline or gradually increase subsequently, then either increase greatly or decelerate at advanced vector age, based on field experiments (Harrington *et al.* 2008), meta-analyses of field data (Clements and Paterson 1981), and laboratory experiments (Dawes 2009, Styer *et al.* 2007a,b). All of these issues are discussed further, below.

Plant-affected components

The following features of vectorial capacity have been shown to be directly affected by plant feeding. The vector density (m) is indirectly affected through sugar feeding's influence both on reproduction (e.g. fecundity and male reproductive capacity) and on survival. The biting rate (a) is affected by sugar directly, through its influence on supplemental feeding within a gonotrophic cycle, its delaying of the primary (ovigenic) blood feeding while the crop contains a large sugar meal, and its delaying of oviposition. Survival (p) depends both on the frequency and quality of sugar feeding. Very little is known about whether energetic reserves affect host choice and the duration of the extrinsic cycle (n). Vector competence (b) is reported to be affected by the inclusion of sugar in mosquito diets, but the manner of the effect seems to depend on the vectors, pathogens, and plants involved. Flight activity and flight range also are vulnerable to plant-sugar availability, and their unfettered performance is an implicit assumption of vectorial capacity, so they also are mentioned below. A related assumption is that host use is random, though evidence suggests otherwise. Finally, a factor in age-dependent models of vectorial capacity is the age at which blood feeding commences (Styer *et al.* 2007a), which relates directly to the blood/sugar feeding choices of young females, discussed under 'Obligatory or facultative nature of sugar feeding'.

Vector competence

Here, we consider vector competence to be the product of the vector's characteristics, which will determine the success rate of a particular parasite to infect that vector, to develop, and then to

be transmitted to the extrinsic host (Hardy *et al.* 1983). There is a gauntlet of challenges parasites must run in order to infect and develop in the vector. Both the efficiency with which they do so and the strength of the challenge they face may be altered by environmental conditions, such as plant-sugar availability and hence energetic reserves of vectors.

The main challenges parasites face in their vectors are exposure to the proteinases and trypsins present in the midgut environment after ingestion with blood. For parasites spending a long period here (such as *Plasmodium* spp.), coagulation of the blood bolus and concomitant formation of the peritrophic matrix interfere with infection. The main bottleneck, however, is associated with invasion of the midgut epithelial cells and passage through the basal lamina to the abluminal side of the midgut. It is at this point that certain *Plasmodium* spp. are subject to melanization (e.g. Chun *et al.* 1995). Further challenges from the insect immune system are faced by parasites during migration through the haemocoel to either the lumen of the salivary glands or the head region (Beerntsen *et al.* 2000). Here we provide an overview of the ways in which plant feeding may affect these processes, in particular focusing on direct toxic effects of plant material on the parasite while it is in the midgut environment, on the effect of energy status on the immune response of the vector, and on the vector's energetic reserves, which serve as a nutrient source for the metabolic demands of the parasite as well as the defenses of the vector. It may be difficult to tease apart whether energy is expended on repair, immune function, or a different drain caused by the parasite.

Toxic effects on *Leishmania* and *Plasmodium*

The most immediate way in which feeding on particular plants could affect vector competence is by an inhibition of the infection by compounds present in the plant. To date, the most compelling evidence for the occurrence of a toxic effect on a parasite resulting from plant feeding is that of mortality and agglutination of *Leishmania major* Yakimoff and Schokhor, the cause of zoonotic cutaneous leishmaniasis, after plant feeding by *Phlebotomus papatasi* Scopoli (Jacobsen and Schlein 1999, Schlein 1986, Schein and Jacobsen 1994). This widespread sand fly inhabits semi-arid regions of the Mediterranean and Middle East and appears to depend on plants, using both their floral nectar and their tissue juices. Some of the host plants provide lectins and toxins. That these cause either agglutination or lysis of the parasites within the sand flies' midguts (Jacobson and Schlein 1999) became evident when sand flies artificially infected with promastigotes were kept for 7 days with access to branches of various plant species or to honeydew secretions. Seventeen percent of females with honeydew had decreased infections, whereas 35-65% with plants had reduced infection loads, compared to a sucrose control. After feeding on *Capparis spinosa* L., *Ricinus communis* L., or *Solanum luteum* Mill. many parasites were agglutinated in clumps and had disintegrated organelles or other aberrations (Schlein and Jacobsen 1994). Extracts of certain plants agglutinated *Leishmania* parasites *in vitro*. The inhibition of this toxic effect in *in vitro* assays was prevented by the presence of various sugars (Jacobsen and Schlein 1999). This result and that of prior studies indicate that lectins prevalent in plants agglutinate promastigotes of *Leishmania* spp. (Davidowicz *et al.* 1975, Dwyer 1974, Jacobsen *et al.* 1982).

Additionally, drought-induced sugar shortages in plants can affect vector population size and parasite survival. With short rasping stylets, the sand flies can cut into healthy plant tissues and ingest sugars, starch granules, and cellulose particles. They generate amylase in their saliva and elsewhere to digest the starch to simple sugars, and the parasites generate both amylase and glucosidase, the latter being capable of both cleaving sucrose and partially digesting cellulose (Jacobson and Schlein 2001, Jacobson *et al.* 2001). The resulting sugars benefit both sand fly and

parasite. During the summer, the plants are stressed by high temperatures and lack of rain, and the plants produce much less sugar. This causes shortened sand fly lifespans, thereby greatly reducing reproduction and also curtailing the probability that a female will become infected by feeding on an infected rodent, survive long enough to allow the parasite to complete its extrinsic cycle, and then transmit it to uninfected rodents. This effect appears to be offset by the natural selection for deprivation-resistant sand flies, which live longer under these conditions. The increased drought tolerance also has the side-effect of weakening the sand flies' ability to eliminate their parasite infections (Schlein and Jacobson 2001).

Vector-produced lectins, proteins that bind with a parasite's structural carbohydrates important for invasion, are implicated in the outcome of several other pathogen-vector associations. For example, in *Ae. aegypti*, addition of N-acetyl-D-glucosamine to a blood meal containing the filarial nematode *Brugia pahangi* Buckley and Edeson facilitated migration of the microfilariae into the haemocoel, apparently because this sugar blocked the action of gut lectins (Ham *et al.* 1991). Unknown at this point is whether sugars with similar effects may be present in plant nectar. Sugar meals have been reported to either enhance or retard the development of malaria and filariasis parasites within mosquitoes (Basseri *et al.* 2008, Kelly and Edman 1997, Pumpuni *et al.* 1996, Samish and Akov 1972, Vaughan *et al.* 1994, Weathersby and Noblet 1973), but these effects and their mechanisms have not been investigated in depth. It also has been reported that exposure of *An. gambiae* to some attractive plant species, before or after an infectious meal, greatly curtails the production of *P. falciparum* oocysts (Manda *et al.* 2005, 2007c, and pers. comm. to W.A.F.). This suggests that, as in sand flies, plant feeding by mosquitoes can affect vector competence directly.

Effects of parasites on sugar feeding (metabolic demands)

Parasitic infection with filarial nematodes or *Plasmodium* spp. reduces mosquito fecundity (Hurd *et al.* 1995), suggesting that mosquitoes harbouring such infections bear a considerable cost. For instance, a reduced egg output as high as 33% has been reported for *Aedes trivittatus* Coquillett infected with *Dirofilaria immitis* Leidy (Christensen 1981). The exact mechanism may be difficult to pinpoint, because the costs can be mediated either by the metabolic demands of the parasite or by the immune response of the vector to the parasite (Tripet *et al.* 2008). In either case, plant-sugar feeding may play an important role in compensating for energetic losses due to infection. An example of a parasite-mediated cost is an increased susceptibility to infection with bacteria following *Plasmodium* ookinete penetration of the midgut epithelium and development of the oocyst between the basement membrane and basal lamina of the midgut. One such bacterium is *Serratia marcescens* Bizio, which mosquitoes may obtain from contaminated sugar wicks in insectaries, which, in conjunction with *Plasmodium* infection, increases mortality strongly (Maier *et al.* 1987). This necessitates efficient midgut repair of invaded epithelial cells. In *An. stephensi* repair begins a few hours after infection with *P. falciparum* through the activation of nitric oxide synthase, resulting in apoptosis or necrosis of the invaded cells, and their subsequent extrusion and replacement. Besides the direct energetic cost, this process uses arginine, a dietary requirement of egg production, thus suggesting a nutrient conflict between fecundity and immune response (Tripet *et al.* 2008).

Evidence that growing oocysts rely on the energetic reserves of the mosquito is scant and mostly indirect. *An. stephensi* Liston infected with *Plasmodium cynomolgi* Mayer have reduced flight capability, as measured by their distance flown, speed, and duration of flight. Furthermore, pre-flight weight between uninfected and infected females was different, indicating that this could be due to differential use of carbohydrate reserves. This is supported by the intriguing finding that

isolated midguts of *An. stephensi* infected with *P. cynomolgi* used up to 8 times more glucose than controls over a 2-hr period (Schiefer *et al.* 1977). Similarly, flight muscles of *An. atroparvus* infected with *Brugia patei* Buckley, Nelson and Heisch were relatively depleted of glucose, and significant incorporation of amino acids by the filarial nematode was observed (Simpson and Laurence 1979).

Several investigators have examined how meals taken by adult mosquitoes prior or subsequent to infectious blood meals affect establishment of infection and development of parasites. Kelly and Edman (1996) provided *Ae. aegypti* with either a sucrose solution or water before an infectious (*Plasmodium gallinaceum* Brumpt) blood meal, and sugar or additional blood meals afterward. Oocyst counts were highest in the group with water before, and sugar after, the infective blood meal, but there were no significant differences in sporozoite load. Infectivity rate was lowest for females that had no access to sugar but had access to additional blood meals after infection, suggesting either that a lack of nutrients from sugar negatively affects parasite establishment or that subsequent blood meals and increased enzymatic activity in the midgut interferes with oocyst development. Vaughan *et al.* (1994), indeed, showed that the accelerated blood-meal digestion resulting from prior blood feeding in *An. gambiae* had a detrimental effect on the production of *P. falciparum* oocysts. Mosquitoes with access only to sugar developed the most oocysts, those with two prior blood meals the least. And this was evident only when ookinete abundance was low. However, when *An. gambiae* was fed blood from human volunteers naturally infected with *P. falciparum*, a different result was found (Okech *et al.* 2004). Mosquitoes that had two prior blood meals (4 days apart) had a higher infection rate than those with one blood meal or only a 10% glucose solution, but oocyst loads did not differ between treatments. Whether females were kept with sugar or just water for the first 2 days after emergence did not affect infection rates. The concentration of sucrose solutions provided to *Culex pipiens pipiens* L. did not affect susceptibility to West Nile virus, but a higher proportion of females transmitted the virus when low-concentration sucrose was available (Vaidyanathan *et al.* 2008).

Effects of energy state on the immune (melanization) response

A robust body of work exists on the insect melanization of parasites, a specialized component of the immune response that sometimes occurs during filarial nematode and *Plasmodium* infections. In a *Plasmodium*-refractory strain of *An. gambiae* (Collins *et al.* 1986) late ookinetes/early oocysts are readily encapsulated and melanized, and negatively charged C-25 Sephadex beads, when injected, elicit a very similar response. Due to the straightforward nature of this method for quantifying the level of response (the proportion of Sephadex beads melanized and the degree of melanization), and its proposed value as a general model for the strength of the immune response (Schwarz and Koella 2002), it can offer precise insights into the interactions between mosquito diet, energetic reserves, and immunity. Additionally, as with midgut repair in response to infection, the involvement of limiting resources besides energy reserves hints at an immunity-reproduction trade-off. For instance, l-tyrosine, an amino-acid precursor of melanin involved with egg-chorion tanning, might be diverted to melanization of a parasite. A case in point is *Armigeres subalbatus* Coquillett, which when challenged with *Brugia malayi* Brug experiences a delay of oviposition (Ferdig *et al.* 1993). Both the aforementioned arginine and tyrosine occur in nectar (e.g. in *Lantana camara* L. (Vrzal *et al.* 2010)), and thus it is plausible that nectar feeding positively mediates such trade-offs.

A number of studies have looked into factors such as age, prior adult diet, and larval nutrition on the efficacy of the adult melanization response, and they are briefly summarized here. In *Plasmodium*-susceptible or refractory laboratory strains of *An. gambiae*, melanization of C-25

beads was highest the day after emergence, and then dropped rapidly. In the two days following a blood meal, melanization was elevated in comparison to non-blood-fed mosquitoes of the same age in the refractory strain, whereas blood feeding had a negligible role in the response of susceptible mosquitoes (Chun *et al.* 1995). In the refractory strain, melanization decreased with increasing temperature and with restricted larval diet (Suwanchaichinda and Paskewitz 1998). In *Ae. aegypti*, melanization was positively correlated with age at pupation and with body size (Koella and Boëte 2002). This contrasts with the finding that *Ae. aegypti* refractory to *P. gallinaceum* had shorter development times and smaller body sizes than susceptible mosquitoes (Yan *et al.* 1997). Possibly, a different aspect of the immune response was involved in the latter study, which would indicate that nutritional status does not uniformly affect the immunocompetence of vectors. Body size did not affect melanization in *An. stephensi*, but adult diet did (Koella and Sørensen 2002). If females had taken a blood meal one day prior to being injected with a bead, the likelihood of complete melanization went up with increased concentration of the available sugar solution. However, if females had not obtained blood prior to injection, the proportion of females that completely melanized their beads did not depend on sucrose concentration. This suggests that to mount an effective immune response, females must feed from a high-quality sugar source to augment the energy and nutrients obtained from a blood meal.

An age-dependent aspect of the melanization-promoting effects of sugar and blood meals also has been found, both in laboratory and field-collected *An. gambiae* (Schwartz and Koella 2002). The strength of the melanization response decreased over the first 4 days of life. For very young females, an increase in glucose concentration increased melanization, but by 4 days this was no longer the case, and instead only a blood meal increased the immune response. Both the proportion of females melanizing beads and the intensity of their response was higher in field-collected specimens. Those with longer wings had a higher likelihood of carrying oocysts, and weak glucose solutions did not affect whether oocysts developed. Few females had melanized oocysts, suggesting that melanization is irrelevant for certain natural vector-malaria interactions. The authors concluded that sugar feeding by *An. gambiae* does not affect the immune response significantly, because young females will rely mostly on teneral reserves and older females on blood meals. However, this may be a premature conclusion if studies in laboratory cages misrepresent energetic expenditures in the field or if females that include sugar in their diet experience energetic increases over multiple gonotrophic cycles.

Clearly, the immune response is energetically costly, and there is limited evidence that this, rather than the metabolic demands of developing parasites, is the main metabolic burden borne by infected vectors. For instance, in the black fly *Simulium ornatum* Meigen infection with *Onchocerca lienalis* Stiles reduced ovarian vitellin contents by half, 36 hr after infection. Because this occurred even at very low levels of infection, it would seem that a costly immune response, rather than energetic drain by the parasite, is competing with fecundity (Hurd *et al.* 1995). Additionally, Rivero and Ferguson (2003) tested whether *Plasmodium*-infected *An. stephensi* females depleted their energetic reserves more rapidly than uninfected females. Levels of whole-body glycogen and lipid did not differ, but glucose amounts were much higher in females with developing oocysts. However, the number of oocysts present did not influence the amount of glucose, suggesting that *An. stephensi* increases its sugar intake when infected, irrespective of the infection load. A cost of infection unrelated to oocyst burden also was found for *An. stephensi* infected with two genotypes of *Plasmodium chabaudi* (Ferguson and Read 2002). Under conditions of sugar deprivation one clone of *P. chabaudi* was more virulent in the mosquito, despite producing a significantly lower oocyst burden than the other clone or a mixed infection. With *ad libitum* glucose, a mixed infection had the highest survivorship cost, and there was no difference between the single infections.

Virulence of different parasite genotypes therefore was concluded to depend on environmental circumstances (i.e. presence of sugar). Lambrechts *et al.* (2006) investigated how *An. stephensi* genotype and environmental conditions (differences in weak glucose solutions exposed on wicks) affected infection with *yoelii yoelii*. Infection rates did not differ among mosquito lines or glucose treatments, but, surprisingly, the number of oocysts was greater with access to 4% glucose than with 2% or 6%. Additionally, infected mosquitoes suffered higher mortality at low glucose concentrations than uninfected females did.

As indicated by the sometimes contradictory findings above, the manner in which sugar intake influences immunocompetence, infection rates, infection loads, and virulence is complex. The main point is that the parasite-vector relationship, and therefore possibly disease transmission in a given area, is highly specific to mosquito genotypes, parasite genotypes, sugar availability, and sometimes to sugar-by-genotype interactions. It is therefore important to place mosquito immune responses in an environmental context, and one aspect of environment (differing both geographically and seasonally) is the presence and abundance of attractive nectariferous plants. Thorough studies on natural host-parasite systems under semi-field conditions will be required before plant-vector-parasite relations can be elucidated and generalized.

Survival

Minor decreases in daily survivorship of adult mosquitoes result in stark declines in vectorial capacity. It is therefore vital to know how survival depends on an environmental factor that has not been taken into account in epidemiological models, namely the composition of the plant community. Few field studies have considered how different nectar sources and their presence affect mosquito longevity; most have been performed in laboratory or semi-field settings, whose results require careful extrapolation to the field. A focus of laboratory investigations has been the difference in survivorship of mosquitoes on diets of sugar and blood or on blood only.

Clements and Paterson (1981) reanalyzed survival data of mosquitoes and concluded that the common assumption of constant mortality throughout life rarely holds. Therefore, McDonald's (1957) malaria model assumption, that mosquito senescence in nature either does not occur or is entirely overshadowed by high daily mortality, also may not hold. Instead, a more realistic value for survival may be derived from a non-linear function, so that the probability of death increases at advanced age.

This is particularly relevant to vectorial capacity, because the age at which a female takes an infected blood meal then affects the probability of her living through the extrinsic incubation period. Models have been proposed that take this into account (Dawes *et al.* 2009, Rasgon *et al.* 2003, Styer *et al.* 2007a). Furthermore, different age-dependent mortality functions produce very different vectorial capacity outcomes. For example, a Gompertz model, where mortality increases exponentially after a certain stage, contrasts sharply with a logistic model, where mortality of older individuals decreases, resulting in a small but highly infective proportion of old mosquitoes (Carey 2001, Dawes *et al.* 2009). While it is established that sugar feeding increases longevity, equally important may be whether mortality of females with sugar in their diet is best described by a function that differs from that of females feeding on blood only. For *Ae. aegypti*, Styer *et al.* (2007a) found that this was not the case. Mortality functions best fit a logistic decline, whether the females had sugar only, blood only, or both. Okech *et al.* (2004) found that female *An. gambiae* kept on blood only senesced faster than females with access to sugar as well.

To date, few studies have delved into the survivorship of mosquitoes when allowed to feed on different natural plants, and these have mostly focused on the malaria vector *An. gambiae*. This is an oversight, given the wealth of knowledge concerning many non-vector taxa on how strongly plants differ in nectar rewards and secondary compounds, and how pollinators respond to plant cues and make use of these resources (Goulson 1999). As a result, basic behavioural investigations on the nectar-foraging behaviour of medically and veterinarily important taxa are just beginning. We do not know whether foraging vectors ignore certain plants, whether responses to plant volatiles are fixed or affected by individual experience, and whether certain vegetation types provide inadequate amounts of nectar.

First steps in this direction were cage studies of *An. gambiae* sugar intake and longevity, comparing cuttings of plant species that occurred in their natural habitat. Gary and Foster (2004) found that overnight females were mostly able to obtain sugar in comparable amounts from honeydew, castor bean (*R. communis*), and cassava (*Manihot esculenta* Crantz), whereas on lantana and on castor bean with the extra-floral nectaries covered there were no sugar-positive mosquitoes. Despite the similar amounts of sugar taken from honeydew, castor bean and cassava, mean survival times differed significantly, suggesting that other aspects of nectar composition affected mosquitoes. In a similar study, only castor bean increased survival to an extent comparable to a 6% sucrose control. Lantana and sweet potato (*Ipomoea batatas* Lam.) resulted in high percentages of sugar positive mosquitoes, but extended survival by an average of only one day over a water-only diet, likely due to the low amounts of sugar obtained. Access to one species (*Amaranthus hybridus* L.) resulted in a slightly worse survival than a water-only treatment (Impoinvil *et al.* 2004).

The sugar- and amino-acid composition of both floral or extra-floral nectar varies among plants and may influence survival of mosquitoes. Yet, how they do so has been studied only in laboratory settings. For example, Vrzal *et al.* (2010) assessed the effect of amino acids on survival of *Culex quinquefasciatus* Say males and females by adding certain amino acids to water or to a mixed sugar solution based on lantana nectar. Although adding amino acids to water did not increase survival, adding these amino acids to the sugar solution increased survival by 5% for females, but not males. Andersson (1992) tested longevity of female *Aedes communis* de Geer of different sizes and with access to different concentrations of sucrose or fructose. No difference in longevity was found between sucrose and fructose solutions, but the mean time of survival was shorter on 10% solutions than on the higher concentrations. Likewise, Fernandes and Briegel (2005) found that *An. gambiae* and *An. atroparvus* survived longest on 5-10% solutions, whereas Briegel *et al.* (2001) reported a linear relationship between sugar concentration and survival in *Ae. aegypti*, with 50% sucrose giving longest survival.

These results strongly suggest that mosquitoes should be discriminating in their plant feeding. That this is indeed the case was demonstrated by observing how frequently mosquitoes rested, probed, and fed on a group of thirteen different plant species in one large cage, and what percentage of mosquitoes had detectable sugar in the crop the morning after exposure to these plants. Based on these parameters, plants could fairly consistently be placed in preferred or non-preferred categories (Manda *et al.* 2007a). When the survival and fecundity of females feeding on these plants was evaluated (Manda *et al.* 2007b), of the plants that were consistently favoured by females, only *Parthenium hysterophorus* Buckl. did not significantly extend survival beyond that of a water-only control. After one blood meal, fecundity of females having fed on *P. hysterophorus* and lantana was less than that of those on the other plants, but after three consecutive blood meals there were no differences in fecundity.

Survival of female mosquitoes and sand flies allowed only blood meals, typically offered once a day, compared to those offered both blood and *ad libitum* sugar solution, has been thoroughly explored for a limited number of species, usually in laboratory cages. Sugar promoted survival of females in nearly every study (recent references: Bowen and Romo 1995b, Braks *et al.* 2006, Canyon *et al.* 1999, Costero *et al.* 1998, Day *et al.* 1994, Fernandes and Briegel 2005, Gary and Foster 2001, Kelly and Edman 1997, Manda *et al.* 2008, Okech *et al.* 2003, Schlein and Jacobson 1999, Straif and Beier 1996, Styer *et al.* 2007a, Xue *et al.* 2008, 2010). In some studies the effect of sugar deprivation on survival may have been exacerbated by restricted access to blood (Okech *et al.* 2003, Souza-Neto *et al.* 2007). Most studies indicate that survival on a mixed sugar-and-blood diet is greater than on sugar alone (Styer *et al.* 2007a, Xue *et al.* 2008, 2010), though Joy *et al.* (2010) found that lifespan was increased by restricted access to blood, so that female *Ae. aegypti* offered one blood meal or none lived longer than females offered blood once a week.

Biting frequency

As noted above, nearly all studies indicate that sugar availability reduces mosquito blood-feeding frequency. The most recent investigations confirm that sugar deprivation tends to promote blood feeding or makes females more responsive to host stimuli or less deterred by repellents (Bowen and Romo 1995a,b, Bowen *et al.* 1995, Braks *et al.* 2006, Canyon *et al.* 1999, Fernandez and Klowden 1995, Gary and Foster 2001, Renshaw *et al.* 1995, Takken *et al.* 1998, Xue and Barnard 1999, 2008). This effect probably is the manifestation of one or more of these phenomena: (a) crop sugar raises the threshold of responsiveness to blood-host stimuli, (b) a large energy reserve depresses the need for supplemental blood feeding during blood digestion, and (c) either crop sugar or a large energy reserve reduces activity and consequently delays oviposition, thus retarding the initiation of each new gonotrophic cycle. The overall result, taken by itself, suggests that the absence of sugar sources increases vectorial capacity. However, all of these studies were conducted in the laboratory, where abnormal amounts and frequencies of sugar ingestion may prolong the interval between blood meals. Field evidence for sugar's depressing effect is equivocal. *Aedes provocans* Walker females with large amounts of sugar generally were at rest, those with moderate meals were biting, and those with small meals were nectar feeding (Smith and Gadawski 1994). Resting *Anopheles freeborni* Aitken more often contained plant sugar than those seeking blood hosts (Holliday-Hanson *et al.* 1997), similar to earlier field work on salt marsh mosquitoes by Magnarelli (1978, 1979, 1980). Female *Ae. aegypti* released after receiving both *ad lib* sugar and replete blood meals completed their gonotrophic cycles 2 days later than those receiving only blood (Morrison *et al.* 1999). An exceptional result was reported by Gu *et al.* (2011), who found that *An. sergentii* in an oasis with a prominent sugar source had shorter gonotrophic cycles over much longer life spans than a population at a similar oasis without that sugar source. A similar conclusion was drawn from field data by Gadawski and Smith (1992). However, in the latter case preliminary sugar meals probably were necessary simply to hasten sexual maturation and initiate the blood-feeding mode.

Energetic reserves resulting from larval feeding may affect biting rates in different ways than those resulting from sugar feeding. For instance, mosquitoes emerging from nutritionally poor larval sites typically are smaller and show reduced host responsiveness. Thus, low energetic levels initially may favour sugar feeding and depress biting rates. On the other hand, small females often require an additional blood meal to bring their ovaries to the pre-vitellogenic resting stage. It has been suggested for both *An. gambiae* and *Ae. aegypti* that energetic deficits are compensated by supplementary blood meals. Such additional, gonotrophically discordant, blood meals increase vectorial capacity. The relative importance of both reduced host-responsiveness and the need for supplemental blood meals thus has implications for the effect of body size on vectorial capacity.

To elucidate, we will review the effects of sugar feeding and energetic reserves on components of biting rate: host responsiveness, pre-vitellogenic and supplementary meals, oviposition delay, and biting persistence.

Host responsiveness

Several studies have investigated the initiation of responsiveness to blood hosts in poorly nourished mosquitoes. Female *Ae. aegypti*, reared on a low diet, showed a weaker response to a host odour than did those on a standard diet (Klowden *et al.* 1988). Access to a 1% or a 10% sucrose solution did not mitigate this weakness. *Aedes bahamensis* Dyar and Knab developed sensitivity to blood hosts faster if they received more nutrition as larvae (Bowen and Romo 1995b). Initiation of host-seeking in *Aedes cantans* Meigen and *Aedes punctor* Kirby was related to accumulated lipid reserves, either through larval feeding or nectar feeding. Whereas *Ae. cantans* would not take a blood meal before 192 hr post-emergence, *Ae. punctor* synthesizes lipids more rapidly and was willing to blood feed after 48 hr (Renshaw *et al.* 1995).

Wing lengths of newly emerged *An. gambiae* were smaller than those of the host-seeking population in the field (Lyimo and Takken 1993), suggesting that a substantial portion of small females dies between emergence and host location, or never expresses host-seeking behaviour. Takken *et al.* (1998) found that responsiveness of large and small females to a human hand in an olfactometer increased similarly over the course of 6 days, but large-bodied females were always more responsive, despite access to 10% sucrose to both size classes. Similar body-size differences in olfactometer performance during attraction to a sugar source one day after emergence have been observed (Foster and Takken 2004). These results indicate that small-bodied females are debilitated by more than just a small energy reserve.

Supplementary blood meals and ovarian development

The taking of multiple blood meals per gonotrophic cycle has most notably been observed and linked to the vector status of *Ae. aegypti* and *An. gambiae* (Beier 1996, Norris *et al.* 2010, Scott *et al.* 1993). Multiple blood meals may be taken by very young gonoinactive females as 'pre-gravid' meals, typically in small females that developed in nutritionally poor larval habitats (Feinsod and Spielman 1980, Lyimo and Takken 1993) or may be taken later in life, either as supplementary meals (e.g. Foster and Eischen 1987) or as primary meals that initiate overlapping gonotrophic cycles (Briegel and Hörler 1993). The influence of teneral reserves and reserves accumulated through sugar feeding on either type of multiple feeding may be different. For instance, female *Ae. aegypti* kept with *ad libitum* sugar show host-seeking inhibition during oogenesis (Klowden and Lea 1979), but this is not the case when lacking sugar (Klowden 1986), as a large proportion of gravid females then continues to seek a host. This depressing effect of sugar is also reflected in biting rates. For example, *Ae. aegypti* kept with only water had a higher biting rate, explained mainly by an increased frequency of supplementary feeding, than did females kept with honey. The opposite effect occurred in *Anopheles quadrimaculatus* Say when kept on water. These females had a supplementary feeding rate almost equal to those on honey, but a higher total biting rate, likely due to the absence of a variable delay in oviposition caused by honey feeding (Foster and Eischen 1987). Supplementary feeding appears to be more common among some tropical anophelines, perhaps used as a tactic to compensate for a smaller blood meal capacity and low teneral reserves, with additional meals increasing fecundity and maternal deposits (Briegel and Hörler 1993). In *An. gambiae*, sugar availability after blood feeding does not inhibit the host-seeking response in the same way as it does for *Ae. aegypti*. While the response to a host was

inhibited for 12 hr following a blood meal, after 24 and 48 hr females showed no sign of inhibition (Klowden and Briegel 1994). Straif and Beier (1996) found that it was only the older females (>20 days) that showed an increased biting rate when kept in the absence of sugar. That was not due to an increase in biting rate with age, but rather to a subgroup of the sugar-deprived females, which fed more often and therefore survived longer.

Female body size does not appear to affect this sugar-dependent biting inhibition, or its absence, prior to oviposition, because gravid females reared under either standard or deficient larval conditions, then kept with sugar, do not respond to hosts (Klowden *et al.* 1988). Thus, either body size has no impact on the tendency toward supplementary blood meals later in life, or the energy deficit required to lower the host-seeking inhibition can be compensated by sugar feeding. This does not rule out the possibility that small females without sugar may take more supplementary blood meals. In the field, Scott *et al.* (2000) found a negative relationship between wing length and blood-feeding rate of *Ae. aegypti* in Thailand, but not in Puerto Rico. Geographic variation in gonotrophic discordance is also apparent among anophelines (Beier 1996, Norris *et al.* 2010). Multiple feeding appeared to be more common in a highland site in western Kenya in *An. gambiae* s.s. and *An. funestus* (14, 11%) than in a lowland site (0, 2%) (Scott *et al.* 2006). The cause of such differences is unknown, but may involve local population adaptation, nutrition, temperature, or all three.

Smaller, nutritionally deprived *An. gambiae* females often require one or two additional blood meals before their primary ovarian follicles reach the (gonoactive) resting stage. It is not entirely clear whether sugar feeding can make up for this nutrient deficit, and how size and sugar feeding influence biting tendencies at this time. Takken *et al.* (1998) showed that despite their lower responsiveness to hosts, small females are in great need of blood. Small females, despite access to sugar, did not reach the resting stage without a blood meal, whereas 52% of large females did. In another study, sugar was able to substitute for a non-vitellogenic blood meal. With sugar, females of all sizes were able to mature eggs with just one blood meal (Fernandes and Briegel 2005).

In summary, while small female mosquitoes may have an increased need for blood meals soon after emergence to make up for energetic deficits, their decreased sugar-and-blood-host-seeking capabilities interfere with this, resulting in a higher probability of feeding on sugar, if sugar is more accessible. Sugar feeding inhibits supplementary feeding in some species, but does not do so in *Anopheles*. Finally, small size may increase the tendency to take supplemental blood meals during later gonotrophic cycles, but this is not yet established.

Delay of oviposition

De Meillon *et al.* (1967) discovered that access to cane sugar resulted in erratic and delayed oviposition by *Culex pipiens*. Likewise, inseminated female *Aedes vexans* Meigen with access to sugar spread their oviposition over a slightly longer period, more often failed to oviposit, and retained a greater number of eggs (Shroyer and Sanders 1977). Increased amounts of egg retention by females given access to sugar after blood feeding also was reported for *Anopheles nuneztovari* Gabaldón (Lounibos and Conn 1991). Klowden and Dutro (1990) found that sugar feeding reduced the responsiveness of *Ae. aegypti* to oviposition-site stimuli and this inhibition was greater at higher concentrations of sugar. This effect was attributed to reduced flight activity of sugar-fed mosquitoes. However, it is plausible that the delayed oviposition when sugar is abundant may be an adaptive response to a favourable energy state, i.e. females with a greater flight range and higher prospects for survival may be more selective in their choice of oviposition

sites and distribution of eggs among them. Females that are close to starvation may readily accept oviposition sites of lesser quality. For example, Tsunoda *et al.* (2010), investigating the effects of body size and sugar access on skip oviposition by *Ae. aegypti*, found that sugar delayed the time between blood feeding and peak oviposition and stretched out the period of egg-laying. Additionally, both larger and sugar-fed females oviposited higher above the water surface and oviposited in a greater number of cups over the course of 8 days. An advantage of this behaviour is that hatching of larvae will be more varied in space and time, a bet-hedging tactic.

Persistence

A special aspect of blood feeding that also is influenced by plant sugar is a vector's persistence at a host. Persistence probably has some bearing on the interval between successful blood meals or infectious bites. Differences in persistence in obtaining a primary blood meal have been detected in mosquitoes that differ in their energetic state as a result of access to sugar. It is not always clear whether this is an effect of sugar in the crop, energy reserves in the fat body, haemolymph, and flight muscles, or all of them. Attack duration declined with repeated attacks by *Aedes triseriatus* Say and *Ae. aegypti*, but it declined more rapidly in females without sugar (Walker and Edman 1985, Nasci 1991). In an *An. gambiae* study that distinguished between persistence (time elapsed before resting on a wall for more than 3 min) and attack number (number of host landings per second), only the number of attacks depended on energy state (Roitberg *et al.* 2010). Females never deprived of sugar had the highest number of attacks. The number of attacks was lowest in those deprived one day and somewhat higher at 2 days, suggesting that feeding tactics vary in a non-linear way with changing energy status. Whether these effects influence biting frequency over multiple gonotrophic cycles remains to be examined.

Reproduction and population density

Effects of sugar feeding and energetic reserves on fecundity

The importance of sugar feeding to a vector's reproduction has been evaluated primarily by laboratory studies, though field experiments are gaining prominence. We follow the definition of fecundity as the number of gametes produced by an individual over its lifetime, and fertility as the number of viable offspring produced (Clements 1992). The latter would be affected by factors such as egg retention, viability, and fertilization, whereas fecundity, which we consider here, is simply the number of mature eggs developed.

Blood meals are used both for vitellogenesis and extra-ovarian reserves (Briegel 1990). The proportion used for oogenesis differs with female body size, suggesting flexibility in the allocation of nutrients. When females are close to starvation or emerge with low teneral reserves, they prioritize synthesizing extra-ovarian reserves. With higher energy levels, a greater investment in vitellogenesis is possible. Increasing lipid reserves by carbohydrate feeding should thus increase egg-batch size of females. Laboratory experiments have demonstrated that female mosquitoes with access to sugar usually produce more eggs per gonotrophic cycle (Briegel *et al.* 2002, Foster and Eischen 1987, Harrington *et al.* 2001, Manda *et al.* 2007a, Mostowy and Foster 2004, Gary and Foster unpublished data). Among first-cycle autogenous species there are exceptions to sugar's positive effect on egg-batch size (e.g. Telang and Wells 2004). The timing of sugar feeding also matters. *An. nuneztovari* that had continuous access to sugar or were deprived of it after blood feeding did not differ in their total fecundity (Lounibos and Conn 1991), indicating that feeding on sugar after a blood meal has little effect on egg production during that gonotrophic cycle.

But some mosquitoes may rely heavily on sugar feeding during vitellogenesis. For instance, *Ae. communis* females lacking access to sugar after a blood meal failed to develop follicles to Christopher's stage V (Andersson 1992).

Directly after taking a sugar meal, a female's distended crop will compete for abdominal space with the midgut's future blood meal, resulting in a smaller blood meal. Because blood-meal size correlates with fecundity, the contribution of sugar feeding to fecundity will depend on the digestion rate and the interval between the sugar and blood meals. This was shown for *Ae. aegypti*, where females with high levels of energy reserves, but with an empty crop, had the highest fecundity, and females with low reserves but a full crop the lowest fecundity. Females with high reserves and a full crop or low reserves but an empty crop had similar, intermediate fecundities. Only in low-reserve females with empty crops was the conversion of blood into eggs considerably less efficient. Thus, in sugar-deprived females fecundity appears to be limited by energy levels, whereas in sugar-fed females fecundity appears to be limited mostly by blood-meal size (Mostoway and Foster 2004).

Fitness

A case has been made, based on the infrequency of sugar feeding and high proportions of supplementary blood meals taken by the anthropophilic species *Ae. aegypti* and *An. gambiae*, that sugar is an inconsequential component of their diet where human hosts are common, and that females optimize their fitness by feeding exclusively on blood. This strict interpretation does not necessarily follow from field data showing low levels of fructose positivity, because sugar may be taken more frequently farther away from indoor sampling sites or during times of stringency. Even if sugar feeding is indeed uncommon in the field, it is not necessarily unimportant: the reproductive success of females that seldom feed on sugar may be higher than that of females that never do so (Ma and Roitberg 2008). Otherwise, it would imply these mosquitoes either are not behaving optimally (i.e. they make mistakes), or the selective pressures toward a blood-only diet are operating, but exclusive blood feeding has not yet reached fixation in certain populations.

Here, we briefly define the measures of fitness that encompass several life history parameters that are affected by sugar feeding. The most commonly used measure of fitness is Fisher's Malthusian parameter, r , the intrinsic rate of increase. For age-structured populations r is obtained by solving the characteristic equation (Roff 1992):

$$\int_0^{\infty} e^{-rx} l(x) m(x) dx = 1$$

where $l(x)$ is the proportion of the cohort alive at age x , and $m(x)$ the production of female offspring at age x . Charlesworth (1994) described this as an adequate measure of fitness in the case of weak selection and random mating with respect to age in density-independent and constant environments. If r is close to zero (the population is stationary), the use of R_0 may be justified. R_0 , the net reproductive rate, is the expected number of female offspring produced by a female over her lifetime:

$$R_0 = \sum_0^{\infty} l(x) m(x)$$

It has been argued that in order for R_0 to be a useful indicator of fitness, the growth rate of the population should not just average to zero, but actually be zero (i.e. it would not be a suitable measure for fluctuating populations) and therefore is better seen as one component of fitness

(Caswell 2002). In environments that are not constant (e.g. alternating 'good' or 'bad' years, or seasons) the geometric mean of the finite rate of increase has been proposed as the most accurate indicator of fitness (Roff 1992).

Whether females of anthropophilic mosquitoes have a greater or lesser fitness with access to blood exclusively, or to blood and also *ad libitum* access to sugar, has been the subject of several studies. An overview of these, and of how including sugar in the diet affects different fitness parameters, is given in Table 1. Most studies report values for daily survival (l_x) and fecundity (m_x), and fitness measures r and R_0 . The only published life-table data for *An. gambiae* s.s. to date reported that both r and R_0 were slightly higher in cages without sugar (Gary and Foster 2001). Braks *et al.* (2006), comparing *Ae. albopictus* to *Ae. aegypti*, found a similar response to the diets in both species. All other studies we are aware of have focused on *Ae. aegypti*. While there is a general consensus that survival is increased when sugar is available, and daily fecundity is decreased, not all studies bear this out. In one, survivorship decreased when females could feed on sugar (Scott *et al.* 1997), and others reported no significant differences between the diets (Canyon *et al.* 1999, Naksathit and Scott 1998). Styer *et al.* (2007b) found no difference in survival when females were housed individually, but an increase with sugar when kept in cages of 200. Harrington *et al.* (2001) found that the outcome depended on the blood source, i.e. with human blood, sugar access made no difference, but with mouse blood, survivorship increased with sugar availability.

The effect of sugar feeding on daily fecundity was mostly negative in these studies, with some exceptions (Day *et al.* 1994, Scott *et al.* 1997). When blood was offered daily, Styer *et al.* (2007b) found no difference in the daily egg production of females offered blood or both sugar and blood. When blood was offered every 2 days, however, females with access to sugar had a significantly lower daily number of offspring. This may have been due to increased reliance on sugar feeding – the infrequency of blood having resulted in missed opportunities to blood feed or the ingestion of smaller blood volumes.

The net reproductive rate, R_0 , was lower for *Ae. aegypti* females with access to sugar in the majority of these studies. Braks *et al.* (2006) and Day *et al.* (1994) reported no difference in this parameter. The only study in which sugar increased R_0 occurred when females were housed in cages together, instead of individually (Styer *et al.* 2007b). Crowding may have increased levels of disturbance, causing more flight activity and energy consumption. Whether those conditions are a better reflection of the stress factors faced in a more natural setting is debatable. The intrinsic rate of increase most often decreased when sugar was available. Costero *et al.* (1998) reported different results for cool and hot seasons in Puerto Rico. During the hot season females feeding only on blood had the advantage, whereas there was no difference in r during the cool season. In crowded cages r was higher when females had daily access to sugar and blood, but, counterintuitively, lower when they had access to blood every 2 days (Styer *et al.* 2007b).

The general impression is that in the laboratory the prolonged life of a sugar- and human-fed female is often insufficient to offset sugar's negative effect on lifetime fecundity in these anthropophilic species. On the face of it, the depressing effect of sugar on long-term fecundity means that natural selection should favour the absence of sugar feeding in these females. This selection should be particularly strong in species lacking a quiescent egg stage (i.e. are unable to accumulate offspring in an 'egg bank' and engage in installment hatching after receiving hatching stimuli), during periods of population growth. Quiescent eggs occur in *Ae. aegypti* and other aedines, but only to a very limited extent in *An. gambiae* and other anophelines. Therefore, anophelines should take full advantage of opportunities for unrestricted reproduction during

Table 1. Summary of published effects of a blood and sugar diet, compared to blood-only diet, on fitness components of mosquitoes.

Study	Species	Setting	Sugar solution % (<i>ad libitum</i>)	Biting rate		Survival (L_x)	Fecundity (M_x)	R_0	Comments
				Blood host	Blood host availability/d				
Straif and Beier 1996	<i>An. gambiae</i>	Glass jar, individually	10% (all ♀s for 1 st 4 days)	Mouse	15 min	↓ (1)	-	-	(1) only for oldest age group
Gary and Foster 2001	<i>An. gambiae</i> s.s.	Cage, individual & pooled	10% (all ♀s for 1 st 2 days)	Human	10 min	↓ (1)	↑	↓ (2)	Vectorial capacity ↓ (1) difference bigger in older ♀s (2) slightly?
Scott <i>et al.</i> 1997	<i>Ae. aegypti</i>	Cage, individual, ambient	10%	Human	10 min	-	↓	↔	↑
Braks <i>et al.</i> 2006	<i>Ae. albopictus</i> and <i>Ae. aegypti</i>	Cage, individually	10%	Human	10 min	↓	↑	↔	Same response for both species
Naksathit and Scott 1998	<i>Ae. aegypti</i>	Cage, individually	10%	Human	10 min	-	↔	↓	Same pattern for large & small ♀s
Harrington <i>et al.</i> 2001	<i>Ae. aegypti</i>	Cage, individually	20%	(1) Human (2) Mouse	15 min	(3)	(1) ↔ (2) ↑	↓? (3)	(3) no statistics provided
Styer <i>et al.</i> 2007	<i>Ae. aegypti</i>	Cage, 200 ♀ + ♂s	10%	Human	(1) 15 min every other day	-	(1) ↓ (2) ↔	(1) ↑ (2) ↑	(1) ↓ (2) ↑
Costero <i>et al.</i> 1998	<i>Ae. aegypti</i>	individually Cage, individually, ambient	10%	Human	(2) 10 min 10 min (?) 10 min	-	↔	↓	NM? (1) cool season (2) hot season
Day <i>et al.</i> 1994	<i>Ae. aegypti</i>	Cage, 200 ♀ + 200 ♂s	2%	Chicken	2 hrs	-	↑ (1)	↔	(1) mean LT_{50} & LT_{90}
Canyon <i>et al.</i> 1999	<i>Ae. aegypti</i>	Specimen vial, 5 ♀, 1 ♂	3% & 10%	Human	10 min, 4x	↓	↔	-	High levels of egg retention, exp. stopped at 12 d

Arrows refer to the effect on parameter by including sugar in the diet compared to blood only; ↓ = decrease; ↑ = increase; ↔ = no difference; - = not measured.

periods of population growth, by feeding only on blood. Yet, they do feed on sugar, both in the lab and in the field.

Perhaps the main critical question about these studies is whether the conditions were representative of those faced by mosquitoes in nature. A mark-release-recapture experiment in Puerto Rico, where female *Ae. aegypti* were kept either with blood only or with blood + sugar for 5 days, then released, did support the general hypothesis that anthropophilic females should rarely feed on sugar (Morrison *et al.* 1999). However, additional validation would be useful, as in this study values for r and R_0 were not obtained, fecundity was assessed for only one gonotrophic cycle, and survival was assessed for just 5 days. Possibly the difference in environment between nature and laboratory cages does not matter, but the higher energetic expenditures and greater risk of mortality associated with real situations – evident even in mesocosm-vs.-cage comparisons (Stone *et al.* 2009, 2011) – could enhance the reproductive success of females that do take sugar. In small spaces, flight energy may be consumed at a lower rate, reducing demand for sugar, yet sugar is encountered incidentally but frequently in cages and ingested after stimulatory contact. Excessive sugar feeding may exacerbate its negative effects on the volume of blood meals, the frequency of blood feeding, and prompt oviposition. More intangible aspects need further study, too, including location of higher quality oviposition sites (Tsunoda *et al.* 2010), mate choice (Stone *et al.* 2011), and maternal effects (Fernandes and Briegel, 2005).

In zoophilic species, whether aedine, culicine, or anopheline, the reduced-survival penalty of relying on blood as the sole source of energy appears to be much greater. Though not well documented, the ability of blood (either human or animal) to sustain life in the absence of sugar appears to be much poorer in these animal-feeding mosquitoes (Fernandes and Briegel 2005, Nayar and Sauerman 1971, Wittie 2003), probably because they are less able to cope with the costs of protein catabolism.

Male insemination capacity and competitiveness

Males often are completely overlooked in studies of nutrition's effects on survival and reproduction. But interest in the biology of male mosquitoes has increased, largely related to concerns about the competitiveness of sterile or genetically manipulated males compared to wild-type males. Experience has shown that competitiveness can be a pitfall for genetic control programs (Reisen 2004).

Due to the commonly held assumption that in polygynous species – in which the operational sex ratio of male to female will be high (Emlen and Oring 1977) – all females will become inseminated, repercussions of male mating behaviour on population dynamics have not been well studied. Assuming that males can inseminate multiple females, regardless of food intake, probably adds to this neglect. A recent review of entomological field studies reports that mating failure of females in nature is common (Rhainds 2010). Often this is age-related (i.e. 'temporary wallflowers' rather than total mating failure), so that less-preferred females take longer to become inseminated, lowering their fitness. A male preference for large female mosquitoes (Okanda *et al.* 2002) and those exhibiting certain qualities or bearing species-specific characters (Hancock *et al.* 1990, South and Arnqvist 2011) has been reported.

Mating failure of mosquitoes in nature is difficult to assess because of the lack of a convenient age-grading method that does not rely on female reproduction. However, because males do not ingest blood, their sexual responsiveness, flight activity, and survival – and consequently their

insemination potential – is completely dependent on reserves carried over from larval feeding and from post-emergence sugar feeding. Hence, changes in sugar availability may lead to pronounced shifts in the operational sex ratio. If male population size declines sufficiently, an Allee effect (i.e. at low numbers, there is a positive relationship between population growth rate and population density) will occur in certain environments or seasons. This requires that the insemination rate of females will drop with declining male:female sex ratio. This will depend largely on the upper bounds of male mating capacity and on the efficiency with which females locate large swarms. Howell and Knols (2009) recently reviewed the mating biology of male mosquitoes and suggested that a typical anopheline male may mate 0-3 times in its lifetime (in monandrous species with a 1:1 sex ratio, which is typical of mosquitoes, the average must be 1), but the maximum probably is higher.

To have even a chance at reproductive success, males must survive through a period of maturation when their terminalia rotate and antennal fibrillae can become erect. Following this they have to engage in the energetically costly and risky behaviour of swarming. In most species this takes place at dusk or both dusk and dawn, though many aedines swarm during the day. Swarming typically lasts for only 10-30 min, but sometimes for hours, especially at higher latitudes. The swarm itself is stationary, with males engaging in a constant to-and-fro, up-and-down movement (Downes 1969), until a female is encountered and clasped. Having sufficient energy to perform this behaviour for multiple nights would clearly favour a male's prospects of mating. Furthermore, a male's mating ability and insemination capacity improves during his first week of adult life (e.g. Verhoek and Takken 1994).

Studies of the effect of body size, an indicator of the reserves a male accumulated as a larva, on mating performance are inconsistent. This may be because on the one hand a larger male is likely to have increased longevity, increased ability to locate a swarm site, and perhaps an increased duration within the swarm (Yuval and Bouskila 1993, Yuval *et al.* 1994). On the other hand, a larger male may have poorer agility in flight and decreased competitive ability to grasp a female (Ng'habi *et al.* 2008). Thus, the ability of male mosquitoes to locate and feed on sugar throughout their lives may be the prime determinant of mating success. Yuval *et al.* (1994) concluded that *An. freeborni* feed on sugar only after swarming in the evening, because only resting males collected in the morning contained significant proportions of fructose, whereas males collected in the late afternoon or during swarming did not. The same appears to hold true for *An. gambiae* (Stone, personal observation) and *Culex tarsalis* Coquillett (Reisen *et al.* 1986). The amount of sugar and glycogen, but not lipids, decreased significantly from the start to the end of swarming. The energetic cost of swarming was calculated to be 0.39-0.51 cal/h, resulting in a consumption of over 50% of available reserves if a male were to swarm for 40 min (Yuval *et al.* 1994). In the case of *Ae. aegypti*, enough males may survive in the absence of sugar to inseminate all females in the same age cohort (Braks *et al.* 2006), at least among mosquitoes that have developed under ideal conditions and are held in small cages. Yet natural selection should always favour males that take sugar meals and thereby greatly increase their mating potential and competitive abilities.

For *An. gambiae* males, successfully mating with a female in the absence of sugar is an almost insurmountable task. Increased teneral reserves and low environmental temperature, causing lengthened survival, increase the odds somewhat. Among sugar-fed males, Gary *et al.* (2009) found that the percentage that erected fibrillae and swarmed increased to almost 100% over the course of 2-3 days, whereas without sugar the percentage doing so was already much diminished by the second day. The proportion of females that were inseminated was influenced by male body size, cage size, and temperature; in small cages sugar deprivation did not affect insemination rates

for the first 2 days of cohabitation, but a smaller proportion was inseminated after 3 days at 23 °C when sugar was absent (at 27 °C all males had died by this time). In a follow-up study, the effect of sugar availability on insemination rates of females in more natural, energetically demanding mesocosms was studied with overlapping cohorts of males and females (i.e. groups of 0-day old males and females were released for 10 consecutive days). Maturing females would therefore have multiple opportunities to mate with maturing males. After 10 days, the cumulative insemination rate of females when sugar was present was 49.7%, compared to 10.9% in the absence of sugar (Stone *et al.* 2009). As mentioned above, directly related to insemination rates is the amount of time a female is likely to remain unmated. If this window between female maturity and time of actual mating is expanded, females will suffer a fitness cost, and this will be absolute if they fail to become inseminated. If this occurs for a significant proportion of females, this may have population-level repercussions. Indeed, simulations of a population projection matrix show just that. When sugar sources are removed from the environment, population sizes are reduced to zero over a wide range of life history parameters (e.g. fecundity), suggesting that *An. gambiae* populations are not viable in the absence of sugar sources (Stone *et al.* 2009). It would be valuable to gain deeper insight into the link between male survival and mating ability and their foraging behaviour. Particularly relevant is how well males are sustained on poor-to-medium-quality sugar hosts, and to what extent males can make up for this by increasing their foraging efforts.

Flight activity and range

Although not a coefficient of vectorial capacity, flight range is important to transmission. This is partly because vectorial capacity makes the assumption that biting will be random within the vertebrate host population. As flight is restricted, chances increase that vectors will bite the same hosts repeatedly, introducing the complications of redundant infections or superinfections. Flight range also is critical to the successful movements of vectors between oviposition sites and vertebrate hosts when the two are widely separated (e.g. Clarke *et al.* 2002). These transigrations must be supported by energy derived either from portions of blood meals not used in vitellogenesis or from plant-sugar meals, as flight-mill studies have shown (e.g. Kaufmann and Briegel 2004). They almost certainly affect two critical vectorial capacity components: blood-feeding frequency and survival.

Learning

The availability of sugar, and therefore its effect on biting frequency and survival, probably changes with vector age as a result of experience. If this conjecture is valid, then sugar's effects on vectorial capacity also change with age. Early experiments with *Cx. quinquefasciatus* and *Cx. pipiens* demonstrated that, by associating plant-produced volatiles with the presence of sugar, young adults became more responsive to them (Jhumur *et al.* 2006, Tomberlin *et al.* 2006). This learning ability is expected to be advantageous in environments where sugar production by different plant species changes seasonally and also would allow adjustment to different plant communities. Non-random selection of blood hosts (McCall *et al.* 2001) and oviposition sites (McCall and Eaton 2001), as a result of experience, also may cause distortions unaccounted for in assessments of vectorial capacity (McCall and Kelly 2002).

Plant-based techniques for vector control and interruption of pathogen transmission

Reducing the incidence of malaria, the deadliest among mosquito-borne diseases, relies on reducing the entomological inoculation rate (EIR), the number of infectious bites received per person per unit of time, usually one year. This relies on the biting rate, a , and density, m , of the vector population, as well as the sporozoite rate, i.e. the proportion with sporozoites in their salivary glands. The main vector control methods used to prevent malaria are currently indoor residual spraying (IRS) and insecticide-treated bed nets (ITN). While great reductions in EIR are often achieved with these methods, the only recent control efforts that have produced an $EIR < 1$, the level required to achieve a sustained reduction in infected-case prevalence, combined ITNs with source reduction (Shaukat *et al.* 2010), i.e. one form of integrated vector management (IVM). The effectiveness of IVM, the WHO-recommended approach to vector-borne disease control (WHO 2004), comes from combining two or more methods that are most efficacious in a particular setting and that complement each other synergistically. Novel control methods that can be used in IVM are urgently needed, and those targeting components of the mosquito life cycle that are left untouched by current methods may be especially promising (Ferguson *et al.* 2010). Because sugar feeding may be exploited for such a purpose, it is all the more poignant that basic knowledge of the feeding decisions and behaviour of even the most important malaria vectors remains scant to date. Here we review promising studies that make use of the sugar feeding behaviour of mosquitoes in control and surveillance of pathogens and vectors.

Marking

Mosquitoes and sand flies can be marked to study their behaviour and survival. One efficient method is to allow the mosquitoes to feed on sugar that has been mixed with a dye or radioisotope. An advantage is that the vectors mark themselves, either at emergence (Reeves *et al.* 1946, Midega *et al.* 2007) or at a suspected host plant (Abdel-Malek 1964, Abdel-Malek and Baldwin 1961, Müller and Schlein 2006, Müller *et al.* 2010b, Schlein and Müller 2008, Schlein and Pener 1990), thus avoiding the disruptive effects of handling. Marking has been effective in experiments on dispersal, flight range, survival, and plant-host utilization. An unfortunate side-effect of field studies in which a marked sugar solution is provided right at the place of adult emergence is that the vectors have an unnatural, easily accessed, and very early source of energy. Therefore, such studies may produce misleading data on the timing of mating, gonotrophic cycle events, early mortality, and average distance flown from the emergence site.

Trapping and surveillance of vectors, and detection of pathogens

Plant-based attractants

Vectors find their sugar sources by volatile organic compounds (VOCs) released from the host plants and by some visual cues. The VOCs appear to be the dominant stimuli guiding mosquitoes from intermediate or long distances to the flowers of their plant hosts or to decaying fruit, whereas visual stimuli are principally the showy white or pale petals that can be detected only within a meter or two. The plants gain by releasing VOCs that serve as attractive signals to pollinators, making the vectors nectar thieves in most cases. It is not yet clear whether vectors are sometimes attracted to specialist-pollinated plants that have nectar inaccessible to vectors. Plant VOCs have not yet been used in surveillance, apart from incidental information gained while evaluating the attractiveness of potential host plants and crude fruit-based attractants (Müller and Schlein 2004,

2006, Müller *et al.* 2008, 2010a, Reisen *et al.* 1986, Schlein and Müller 2008). An effective field-tested synthetic odour blend, based on a plant's natural VOC headspace, remains to be developed.

The advantages of using phytochemical lures in traps for mosquitoes, and major hurdles that must be cleared, have been reviewed and described in detail in Foster and Hancock (1994) and Foster (2008). The main advantages are (1) attraction of both males and females of all ages of nearly all species of mosquitoes, (2) early detection, because sugar feeding is often the first activity after emergence, and males tend to emerge first, (3) localization of emergence sites, because males tend to remain more localized, (4) attraction of females in all gonotrophic states, not just those in the blood-seeking mode, and (5) attraction of females in reproductive diapause, when they will not seek blood. In other words, plant-volatile baited traps would target a much wider segment of a mosquito population than is typically sampled with CO₂-baited traps and ovitraps and would not be so seasonally restricted in temperate zones.

Because plant-sugar feeding usually occurs in the same general activity period as blood-host seeking, there is likely to be competition between the two resources, and it is generally thought that blood-host volatiles will be dominant over floral volatiles. However, during early life this may not be the case, and the relative strengths of the volatiles do matter. An appealing option is to combine phytochemicals with vertebrate kairomones, possibly gaining the advantages of each. But whether these volatiles would be additive, synergistic, or mutually inhibitory remains to be tested. The combination of phytochemicals with oviposition-site volatiles in gravid traps also might be effective (females may prefer to oviposit near sites where they and their offspring can quickly regain energy) and is worth considering.

The major hurdle is then simply the identification and synthesis of a plant-volatile blend that is attractive to mosquitoes at a high release rate so that it can out-compete naturally occurring plant odours. A complication is that relatively little is known about the attraction of mosquitoes to specific plant volatiles. An example of this is that the chemical cues coming from extrafloral nectaries of host plants and from host plants that must be pierced to obtain sugar, are almost completely unstudied. The latter clearly are attractive at a distance to the sand flies that feed on them (Junnala *et al.* 2010, Müller *et al.* 2011, Schlein and Müller 1995, Schlein and Yuval 1987). And Schlein and Müller (2008) reported that branches of similar plants with or without honeydew did not differ in the numbers of mosquitoes attracted, suggesting that honeydew itself is not attractive. Honeydew, though readily fed upon, appears not to be attractive to sand flies either (Müller and Schlein 2004, Müller *et al.* 2011). However, sand fly attraction to an aphid alarm pheromone has been demonstrated and may act as a cue to the presence of honeydew (Tesh *et al.* 1992).

Therefore, mosquitoes discover sugar by tarsal chemoreception when resting on plants, or they may be attracted to general plant volatiles and locate the nectaries by random walk. However, activation and orientation of mosquitoes towards floral VOCs are indisputable, and strong attraction to flowers of particular plant species has been shown. For instance, in a small bioassay chamber probing response of *Ae. aegypti* to extracts of milkweed (*Asclepias syriaca* L.) was greater than to extracts of Canada goldenrod (*Solidago canadensis* L.), the former being the more fragrant flower (Vargo and Foster, 1982). And there is upwind attraction of *Ae. aegypti* to isolated floral odours of ox-eye daisy (*Leucanthemum vulgare* Lam.), but an extract did not elicit landings (Jepson and Healy 1988). A role for floral odours in the mediation of upwind nectar-source location also was demonstrated by the attraction of *An. arabiensis* to an extract of *Achillea millefolium* L., a temperate plant. The major component of the odour was reported to be a cyclic

or bicyclic monoterpene (Healy and Jepson 1988). Mauer and Rowley (1999), using *Cx. pipiens*, confirmed that milkweed flowers are attractive in an olfactometer, but synthetic blends they created were not. By contrast, both individual components and synthetic blends of *Silene otites* (L.) Wibel headspace were found to be attractive to the *molestus* form of this species (Jhumur *et al.* 2006, 2007, 2008). Of 36 odour-receptor neurons on type A2 sensilla trichodea of female *Cx. pipiens*, 19 were relatively specific to bicyclic monoterpenes containing a ketone group (thujone and verbenone). The other 17 sensilla were more broadly tuned, and also were sensitive to other compounds, such as green-plant odours. Upwind flight was not elicited by exposure to each of these terpenes alone, or in combination with CO₂, suggesting that an odour blend is needed for plant location (Bowen 1992b). Carey *et al.* (2010) found that individual odourant receptors of *An. gambiae* that had strong responses to esters and aldehydes, volatiles common in the headspace of fruits, were all broadly tuned. Their role in discrimination between such volatiles is not entirely clear, but narrowly-tuned receptors often appear to be associated with salient, i.e. ecologically highly relevant, odours. A comprehensive study on the breadth of odourant sensitivity to volatiles present in attractive floral headspace has not yet been performed.

If mosquitoes have strong preferences for certain volatile blends, baits may be able to out-compete natural sources, unless nectar sources are very abundant. In recent years, more information on the specific attraction of certain plants and sugar sources has come from studies in Israel and Mali. A general impression is that there are certain super-attractants, and even natural concoctions based on these sources are effective in sugar baits. Their use, in combination with insecticides, is discussed below.

Salivation to detect pathogens

Mosquitoes and sand flies salivate while feeding on sugar, both to break down oligosaccharides with α -glucosidase – and perhaps also to break down starch with amylase – and to dilute very concentrated sugar solutions to facilitate ingestion. While salivating, they release viruses and malaria sporozoites (Beier *et al.* 1991, Billingsly *et al.* 1991, Russell *et al.* 1963, Van den Hurk 2007). Van den Hurk *et al.* (2007) tested whether this made it possible to determine infectivity rates of vectors without removing and testing salivary glands or testing extracts of whole insects. After mosquitoes were fed on a blood/virus mixture and provided with sucrose-soaked cotton pledgets after an extrinsic-cycle period, viral RNA was detected in the pledgets by RT-PCR. To see if this could be used as a convenient monitoring system, CO₂-baited updraft box traps were deployed in the field, in which mosquitoes would feed on honey-soaked cards that preserve nucleic acids (Hall-Mendelin *et al.* 2010). These cards were collected once per week and presence of viruses successfully detected. The main advantage of this method over previous ones is its ease, because only the cards need to be tested for virus, which then can be associated with the species of mosquitoes within the trap, even after the mosquitoes have died and their viral contents corrupted. This method speeds up the turn-around time, improving early-warning systems.

Reduction of population density and age by deploying toxic sucrose solutions

Treatment of resting sites, including vegetation

When combined with a 20% sugar solution, Lea (1965) found that surface application of malathion, used as a residual insecticide, killed *Ae. aegypti* mosquitoes at one-tenth the dosage required otherwise. This effect was apparently because the irritancy of the mixture was offset by

its gustatory stimulation, so that mosquitoes remained in contact with it longer and perhaps also fed on it. The duration of its effectiveness also was extended considerably.

Over recent years, a control method that cleverly exploits sugar feeding has been developed and tested in several different environments with several species of mosquitoes. Attractive toxic sugar baits (ATSB) employ fruit scents to attract both male and female mosquitoes, a sucrose solution to stimulate feeding, and an oral insecticide – either boric acid or spinosad, both having very low vertebrate toxicity. Theoretically, the development of resistance can be avoided by rotating among many oral insecticides, although evolution of behavioural resistance (e.g. avoiding sugar sources) is a potential concern.

That this technique is effective in arid areas with relatively few flowering plants was demonstrated by spraying *Aristida raddiana* Savi (the only local flowering plants at the time) with dyed toxic sugar solution in a small oasis in Israel. In the control site, where the same solution was applied minus the toxin, between 80-90% of *An. sergentii* and 72-86% of *Ae. caspius* Pallas were marked with the dye, indicating that they rely predominantly on this plant species for their sugar. Both populations were eliminated in the treatment oasis (Müller and Schlein 2006). In another study, *Cx. pipiens* was shown to be strongly attracted to flowering *Tamarix jordanis* Boiss. branches. Treatment of just this one species with toxic sugar reduced *Cx. pipiens* numbers tenfold, although after 18 days the population rebounded (Schlein and Müller 2008). Similarly impressive results were obtained when the attractant was the juice of rotting nectarines and red wine, when sprayed on vegetation surrounding a sewage pond (Müller *et al.* 2010c). Further examples of the potential of this technique were demonstrated by applying boric acid in sugar solutions on the leaves and stems of vegetation in outdoor screen cages as well as smaller cages, which resulted in significant mortality of *Ae. albopictus* and *Cx. nigripalpus*, as well as a reduction in human landing rates. But *Aedes taeniorhynchus* Wiedemann was apparently unaffected by the boric acid solution (Xue *et al.* 2006). Even sub-lethal exposures reduced host seeking, fecundity, and survival of *Ae. albopictus* (Ali *et al.* 2006).

Perhaps the most relevant question pertains to the applicability of this method to *An. gambiae* s.s., not only because it is one of the prime vectors of *falciparum* malaria in sub-Saharan Africa, but because the reliance of females on sugar in the field is a subject of debate. A field trial in Mali suggests we should be hopeful, because mosquito abundance dropped by 90% following the spraying of guava and honey-melon based ATSB on patches of vegetation of unknown attractiveness surrounding larval sites, and the percentage of females reaching at least 3 gonotrophic cycles dropped from 37 to 6% in the treatment area (Müller *et al.* 2010b).

Overall, these results suggests toxic sugar baits may be highly effective in semi-arid areas of Africa, especially where breeding sites are spatially segregated from domestic areas. The method is cheap and easy to implement, the attractant is easy to produce, and the approach can be used synergistically with ITNs.

Attractive toxic bait stations

As an alternative to spraying toxic sugar solution on vegetation, the use of sugar feeding stations also has been developed and tested. These consist of soda bottles filled with the solution (overripe nectarine juice, wine and sugar, and an oral insecticide), with a hole cut into them through which a wick keeps a sock wrapped around the bottle moist. A hood is placed atop this construction to shield it from the elements (Müller and Schlein 2008). In one study, these were placed at

the openings of cisterns, the resting and larval development sites of *An. claviger* Meigen. After introduction of the baits the number of females in that area decreased ten-fold (Müller and Schlein 2008). These bait stations also were used in a study in oases, this time suspended from *A. raddiana* trees. Baits that were laced with an insecticide steadily reduced the *An. sergentii* population to less than one tenth, and *Ae. caspius* to one third of the starting population (Müller *et al.* 2008). It is not immediately clear why the results, while impressive, were not as dramatic as the 2006 study, when full elimination was achieved. It may indicate that the bait stations are less effective than the spraying of resting vegetation.

Whether this station-based ATSB approach will work when they are placed in houses with vertebrate hosts, in lush areas with greater competition of natural nectar sources, or in urban areas where humans and breeding sites are closer together, remains to be seen. And, its impact on EIR or malaria prevalence has not been studied. A principal concern in the application of ATSB is its impact on non-target sugar feeding insect orders, such as Hymenoptera, Lepidoptera, Coleoptera, and Blattaria. This is unlikely to be an issue when applied within houses, where elimination of ants and cockroaches is welcome, and risk to humans is negligible. But outdoors, lethality to such beneficial insects as parasitoid wasps and various pollinating bees, moths, beetles, and other dipterans, requires special consideration. A partial way around this obstacle is to design attracticide stations that allow access to mosquitoes and other vectors but exclude large-bodied pollinators. An alternative is to provide a toxin whose action is specific to nematoceros dipterans or to the pathogens they carry.

Selective plant removal or replacement

A potential alternative use of plant feeding to control vectors, not involving insecticides, is the selective removal and replacement of their principal sources of sugar (Abdel-Malek and Baldwin 1961, Abdel-Malek 1964). In mesocosms, removal of sugar sources causes a dramatic reduction in *An. gambiae* reproduction, to the point of creating an environment that cannot sustain the population (Stone *et al.* 2009). To be practical and to have a minimal impact on the environment, this approach requires that the host plants be both few in diversity and low in density. Obviously, if a vector can use any of 5 or 10 different plant species in a transmission zone, and at least some of them are abundant, their removal and replacement would be onerous (Schaefer and Miura 1972), except close to human habitations. To identify opportunities where this approach is feasible, we first must overcome the obstacle of determining innate and learned plant-host breadth. Following removal of sources with highly attractive cues, will the ability of mosquitoes to efficiently locate nectar be significantly diminished, or will the mosquitoes simply shift to the next best plant?

One topic that will be important to plant-based control is the contribution of homopteran-generated honeydew to sugar meals of vectors. If honeydew is a large part of the sugar diet, plant removal to diminish sugar meals will require identifying the homopteran's plant hosts. The few studies that have attempted to quantify this suggest large differences between mosquito species (Burkett *et al.* 1999, Russell and Hunter 2002). This subject is understudied, and more data are needed to make generalizations about honeydew's importance according to vector species and locality.

A different tactic is suggested by the occurrence of plants that are naturally toxic to vectors but that are nevertheless attractive to them. The ornamental *Bougainvillea* has this effect on *Ph. papatasi*, and circumstantial evidence indicates that in its vicinity sand fly numbers are much lower than in other locations (Schlein *et al.* 2001). Toxic species, if planted around human

habitations, might provide sustained natural suppression of vector densities and lower survival, thereby compromising their vectorial capacity. Such plants have not yet been discovered that work against mosquitoes or other vectors.

Inoculation with microorganisms

Sugar baits have been used to induce *Cx. pipiens* mosquitoes to pick up the pathogenic bacterium *Bacillus sphaericus* and transfer it to larval development sites (Schlein and Pener 1990). Additionally, attention has been drawn to symbiotic gut bacteria that interfere with the ability of ingested mature *Plasmodium* gametocytes to form oocysts in the gut wall of *Anopheles* (Pumpuni *et al.* 1996). Attractive and palatable sugar baits have been tested as a means of spreading such bacteria into the mosquito populations (Lindh *et al.* 2006). Acetic-acid bacteria (Acetobacteraceae) are acquired naturally in nectars, fruit sugars, and phloem sap (e.g. Crotti *et al.* 2010, Suzuki *et al.* 2010, Yamada *et al.* 2000), invade most relevant organs of the mosquitoes' bodies, and are transmitted both horizontally and vertically within mosquito populations. Such bacteria show promise for generating anti-parasite molecules within vectors (Damiani *et al.* 2008, Favia *et al.* 2007, Riehle and Jacobs-Lorena 2005, Riehle *et al.* 2007). One candidate is the osmotolerant bacterium *Asaia*, strains of which can infect the major vector species of mosquitoes (Chouaia *et al.* 2010) and can be transformed easily with foreign DNA (Favia *et al.* 2007, 2008) to produce strains that inhibit malaria development, thereby eliminating its vector competence.

Conclusion

The aim of this review is to raise awareness of the potential effects vegetation abundance and composition may have on pathogen transmission dynamics. This overlooked aspect of the biology of mosquitoes shows tremendous promise for novel surveillance and control methods. Parallels between relevant sand fly and mosquito studies draw attention to gaps in the latter. Uneven knowledge of the topic and contradictory results currently frustrate our attempts to form a holistic view of the epidemiological consequences of sugar feeding in mosquitoes. Here, in summary, are four major questions in need of further research: (1) What is the nectar-host breadth of vector species? (2) What properties make certain plants attractive? (3) How is vector performance affected in the absence of highly attractive, nectar-rich plants? and (4) How do stimulus strength and perceived quality of plant and vertebrate volatiles interact to mediate host choice? Addressing these questions not only for anthropophilic species, but also for generalist and zoophilic mosquitoes, would broaden our current view of mosquito-nectar dynamics, which relies excessively on experimentation with *An. gambiae* s.s. and *Ae. aegypti*. Further, the use of natural systems would greatly improve our understanding of sugar's effect on vectorial capacity and reproductive success, by demanding more realistic energy expenditures. The best systems would include not only semi-field environments, but also wild-type mosquitoes, natural vector-parasite interactions, and natural blood hosts.

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